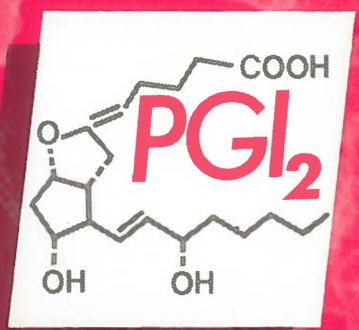
j.j.a.m. van den dungen




# PLATELET PRESERVATION DURING CARDIO PULMONARY BYPASS

# PLATELET PRESERVATION DURING CARDIOPULMONARY BYPASS

# STELLINGEN

I.

Een doorbraak in het gebruik van kunststoffen voor het vervaardigen van kunstorganen, kan alleen plaatsvinden als een beter begrip wordt verkregen van de interactie tussen bloed en kunststoffen.

II.

Toediening van medicamenten die de thrombocyten-functie remmen tijdens het gebruik van de Hart Long Machine, kan resulteren in een beter behoud van de thrombocyten aantallen en functie.

III.

Medicamenten die de thrombocyten-functie remmen, kunnen heparine tijdens het gebruik van de Hart Long Machine niet vervangen.

IV.

Bij hart operaties verdient het gebruik van de membraan oxygenator de voorkeur boven die van de bubble oxygenator.

V.

Aangezien voor de gaswisseling in membraan oxygenatoren de Wet van Fick geldt, heeft turbulentie van bloed ook voordelen.

VI.

Bij de keuze van behandeling van het inversie letsel van de enkel, dient ook het „tapen" overwogen te worden.

VII.

Endoscopische biopsien bij patienten met alkalische reflux gastritis na maagresectie, dragen niet bij tot het bevestigen van de diagnose, noch tot het vaststellen of de chirurgische therapie succes heeft gehad.

VIII.

Vaatprothesen van resorbaar materiaal kunnen een alternatief worden voor vene-transplantaten.

IX.

Uitslagen van echografisch onderzoek dienen met enige reserve geïnterpreteerd te worden.

X.

Indien op een fietspad verkeer in twee richtingen is toegestaan, dient dit aangegeven te worden voor het verkeer op de kruisende wegen.

XI.

Rozen kunnen beter in het voorjaar, dan in het najaar gesnoeid worden.

Stellingen
behorende bij het proefschrift van
J. J. A. M. van den Dungen
Platelet preservation during cardiopulmonary bypass
Groningen 1983

RIJKSUNIVERSITEIT TE GRONINGEN

# PLATELET PRESERVATION DURING CARDIOPULMONARY BYPASS

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. L. J. Engels
in het openbaar te verdedigen op woensdag 8 juni 1983
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Johannes Josephus Antonius Maria van den Dungen

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GRONINGEN

Promotor : Prof. Dr. Ch. R. H. Wildevuur
Co-promotor : Prof. Dr. J. N. Homan van der Heide
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*Aan Nies*

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# CONTENTS

# INTRODUCTION

The technique of cardiopulmonary bypass (CPB) to support the patient during cardiac surgery can nowadays be performed safely. Blood transfusions are needed mainly to replace post-operative blood loss that is attributed to an impairment by CPB of the hemostatic capacity. Although Jehovah's Witnesses have been operated upon without the use of any donor blood, in many centers more than five donor units of blood are needed for each heart operation[1-4]. This may explain the occurrence of post-transfusion hepatitis reported in patients operated upon for cardiac disease[5].

Severe post-operative bleeding has generally been experienced in about 4% of open heart operations[6,7]. The incidence of excessive bleeding due to coagulation defects is 1-2%. However, the incidence of excessive bleeding depends on the investigator's definition of excessive bleeding and the period during which it is measured. Major coagulation defects can also occur without excessive bleeding. Excessive bleeding due to surgical causes usually occurs without major coagulation defects.

The following authors have categorized the factors contributing to excessive bleeding.

Bachmann et al.[8] studied the coagulation factors and fibrinolysis in 512 patients after CPB. Despite inhibition of the clotting cascade by heparin and subsequent neutralization of the heparin, major disorders of coagulation were found post-operatively in 29 patients (5.6%). However, only four of these patients bled excessively. The most common abnormalities were low prothrombin times (PT) and impaired whole blood clot retractions. A discrepancy between low PT's and normal or only slightly depressed factor II, V, VII and X activities was explained by an inhibitor of the extrinsic system. Eight patients exhibited a heparin rebound phenomenon. Hepatic dysfunction was found in four patients, and severe depression of vitamin K-dependent factors due to oral anticoagulation in two. Only two patients had disseminated intravascular coagulation. Seventeen patients with normal coagulation profiles bled excessively. Revision of the wound in 13 patients revealed a surgical cause for the bleeding. Although platelet number and function was not determined in most of these patients, two of the patients with excessive bleeding had thrombocytopenia and marked platelet dysfunction. In two patients the cause of the bleeding could not be determined.

Lambert et al.[2] studied post-perfusion bleeding in 884 patients. Hyper-

fibrinolytic bleeding occurred in 159 patients (20%). These patients were treated with epsilon-aminocaproic acid. Three patients had residual heparin (0.4%). Five patients had normal coagulation studies but bled excessively. These patients required immediate reexploration for surgical bleeding.
Moriau et al.[9] found a moderate decrease of factors I, II, VII, X and XII, but also found a drop in factor V. No evidence was found for a process of disseminated intravascular coagulation.
Kalter et al.[10] found also a significant decline of factor I, II and VII to XII, but the decline of factor V was most pronounced. He suggested a minor role for increased fibrinolysis or disseminated intravascular coagulation.

Many of the reports related to the increased bleeding tendency after CPB are focused on platelet damage. Study of the course of platelet number and function reveals the following. During CPB the platelet count drops to between 30 and 50% of the pre-bypass level[10-14]. A slight recovery is usually seen in the first hours following bypass. The platelet count remains at this low level for 2-3 days. Thereafter the platelet count increases and returns to the pre-operative level on the 6th or 7th day. Platelet counts increase up to the 12th day and return once again to the normal value between the 15th and 20th day.
Although the course of the platelet numbers has been extensively studied by several investigators, only limited information is available on the course of platelet function[10 11 13 15]. Platelet adhesiveness falls precipitously at the onset of CPB, remains low during perfusion and then sometimes returns to the normal range shortly thereafter, but usually not before the third post-operative day[14]. Platelet aggregation with ADP, adrenalin and collagen decreases during CPB and increases again after the third post-operative day, reaching pre-operative levels on the sixth day and maximum values on the thirteenth day, after which it declines to normal. Platelet factor 4 ($PF_4$) levels in plasma increase during CPB and on the following days[9]. The post-operative bleeding time is usually markedly prolonged.
Factors which contribute to the thrombocytopenia and platelet dysfunction include *hemodilution, shear stresses, platelet-surface interface, platelet-gas interface, heparin* and *protamine.*
The decrease in platelet number by **hemodilution** depends on the volume of clear prime used in the extracorporeal circuit. Priming volumes range from 1.5 to 3 liters. Thus a decrease of about 30 to 50% is attributable to hemodilution. The measured decrease is 50 to 70%, but the actual platelet

loss may even be greater because there is a rapid release of platelets from the splenic pool, which houses about 30% of the total platelet population. CPB without hemodilution in splenectomized dogs causes an immediate decrease in platelet count to about 30% of the pre-bypass value. It is known that during CPB, platelet aggregates are sequestered in various sites, the major site being the liver. The rebound of the platelet count immediately after CPB has been attributed to the dissolution of platelet aggregates and the release from their sites of sequestration[16].Some priming solutions such as dextran have a direct influence on platelet function[17].

The platelet loss by hemodilution can be reduced by using devices with small priming volumes and by using platelet-compatible priming solutions.

Shear stresses are generated by the cardiotomy suction, pumps, connectors, geometry of the oxygenators and high blood flow rates. These factors induce turbulence of the laminary blood stream which causes destruction of platelets.

The cardiotomy suction is an autotransfusion system where the blood lost in the thoracic cavity during heparinisation is returned into the circulation. Usually large amounts of air are aspirated with the blood. This is called uncontrolled suction.

Shear stresses and resultant platelet damage can be reduced by the use of controlled cardiotomy suction, non-occlusive pumps and well-designed oxygenators that are efficient at low flow rates.

The platelet-surface interface is actually an interaction of platelets with plasma proteins adsorbed on the synthetic surface. The nature of the foreign surface determines the type of plasma proteins that are preferentially adsorbed, and the type of proteins influences the extent of platelet adhesion[18]. The protein layer has a depth of approximately 20 nM and is composed largely of fibrinogen, gamma-globulin, albumin and prothrombin. Adsorption of coagulation proteins, factor XII, pre-kallikrein and kininogen occurs with ensuing activation of the intrinsic pathway of coagulation[21]. Platelet glycosyl-transferase interacts with a glycoprotein acceptor adsorbed to the foreign surface. Platelet adhesion is primarily an enzymatic mechanism, but some physical adhesion may be involved. The adherence of platelets results in a decrease in the platelet count, a decrease which is proportional to the area of the synthetic surface. The adhered platelets will become detached within thirty minutes. Measurement of their $PF_4$ contents indicates that these platelets have undergone extensive release of platelet contents and are incapable of aggregation and renewed release[20]. Improve-

ments of the platelet-surface interaction can be obtained by using hemocompatible materials such as silicone rubber. Furthermore, selective adsorption of more albumin than gamma-globulin or fibrinogen should result in less thrombogenic surfaces[18 21-23].

The **platelet-gas interface** is the dominant interaction in the bubble oxygenator and during uncontrolled cardiotomy suction. In an oxygenator, oxygen has to diffuse to the red blood cells. The oxygen in a bubble oxygenator is in direct contact with the blood. By the formation of a continuous bubble stream, a large blood-gas interface and turbulence are created.

Gas nuclei, persisting on the synthetic surfaces after the priming of the circuits, are a third source of platelet-gas interaction[24]. Platelet damage caused by the direct blood-gas contact can be reduced by using membrane oxygenators, controlled cardiotomy suction and subjecting the material to a vacuum[24-26].

**Heparin** inhibits the activation of the coagulation system, but does not prevent activation of the platelets. To the contrary, heparin and **protamine** can induce platelet aggregation[27]. Heparin and protamine should be given only in dosages relevant to a quantitated inhibition of the coagulation system. However, no reliable method is available for a quick measurement of the heparin induced anticoagulation. Therefore, more heparin and protamine is given to the patient than is required.

Platelet activation can start with aggregation or adhesion. Aggregation induces adhesion and vice versa. Both lead to release of platelet contents. Whether aggregation or adhesion dominates depends on the type of activator. Shear stresses, platelet-gas interface, heparin and protamine will lead primarily to aggregation immediately followed by adhesion, while the platelet-circuit interface induces adhesion followed by aggregation. However, these sequences are largely hypothetical. It must be assumed that these basic platelet reactions occur nearly simultaneously during CPB and that none of these reactions can be prevented without interfering with the others.

Platelet activation can be reduced by the above mentioned improvements, but some activation will still persist in the unphysiological extracorporeal circuits, leading to loss of platelet number and function. Loss of platelet function is thus a major contributor to the problem of post-operative bleeding, particularly when complicated by disorders of the coagulation system or poor surgical hemostasis. A further improvement has therefore

been attempted by the pharmacological inhibition of platelet activation. The interest to inhibit platelet activation during extracorporeal circulation can be noted since 1971[28]. Initial research was directed to an agent which specifically could inhibit platelet surface interaction but at the same time would preserve platelet hemostatic capacity. This agent would help prevent bleeding in the heparinized patient during long-term extracorporeal oxygenation in the treatment for respiratory insufficiency.

The basic reactions during activation of platelets are adhesion, aggregation and the release of platelet contents. Adhesion occurs to most synthetic surfaces. Aggregation can be induced by the release of platelet contents after adhesion or after exposure to ADP, collagen, thrombin or epinephrine. Most aggregation-inducing agents stimulate phospholipase, which causes release of arachidonic acid from the phospholipid pool of the platelet membrane[29]. Cyclo-oxygenase converts arachidonic acid in endoperoxides ($PGG_2$, $PGH_2$), which can be metabolized to thromboxane $A_2$ ($TXA_2$) by thromboxane synthetase[19]. $TXA_2$, collagen and thrombin can directly induce release. An intracellular calcium ($Ca^{++}$) flux may be essential in platelet activation leading to release. Stimulation of platelet adenylcyclase for the conversion of ATP to cyclic AMP is essential for inhibition of platelet activation. Phosphodiesterase metabolizes cyclic AMP to AMP, which has no anti-aggregatory activity. Most of the pathways of platelet metabolism can be stimulated or inhibited. Some of the drugs with an inhibiting effect on the platelet function also influence other pathways.

$PGE_1$, $PGI_2$, dipyridamole (Persantin®) and sulfinpyrazone (Enturen®) are well known platelet function inhibiting drugs. Sulfinpyrazone is a cyclo-oxygenase inhibitor. Dipyridamole inhibits phosphodiesterase. $PGE_1$ and $PGI_2$ stimulate adenylcyclase. Comparison of the in vitro platelet function inhibiting effects shows that $PGI_2$ and $PGE_1$ are the most potent platelet function inhibiting drugs, followed by dipyridamole and sulfinpyrazone. The platelet release reaction is most effectively inhibited by sulfinpyrazone, followed by $PGE_1$ and dipyridamole. Platelet adhesion is inhibited by $PGI_2$, $PGE_1$ and dipyridamole. $PGI_2$ prevents aggregation at much lower concentrations than those required to prevent adhesion[29]. These effects are strongly dose dependent, and in clinical application the dose will be restricted by side-effects.

Mielke et al.[30] stated that the ideal agent would effectively alter the platelet-surface interaction without causing impairment of the platelet function. Thus, drugs that primarily inhibit platelet adhesion would be preferable.

Braun et al.[28] administered dipyridamole to dogs during extracorporeal circulation and for 5 days thereafter, measuring platelet count throughout this period. They found that dipyridamole improved the number of circulating platelets.
Another approach to prevent platelet adhesion was controlled release of platelet function inhibiting drugs from synthetic surfaces in monolithic delivery systems[23 31]. However, no drug appeared to be selective enough to prevent the platelet-surface interaction and at the same time preserve the platelet's hemostatic capacity.

In summary the problems encountered are:
— CPB disturbs hemostasis. This can be associated with the problems and risks of severe hemorrhage and subsequent transfusion of large amounts of donor blood.
— platelet activation persists despite the use of heparin and despite improvements of the circuit.
— platelet function inhibiting drugs used so far are not specific enough to inhibit the activation of the platelets by the extracorporeal circulation and maintain platelet hemostatic capacity at the same time.

The purpose of this study is to investigate whether complete but immediately reversible pharmacological inhibition of platelet function, with intrinsic temporary loss of hemostatic capacity during CPB, results in better maintenance of platelet numbers and preservation of platelet function. This requires a platelet function inhibiting drug which is intravenously effective, has an immediate action, a strong effect, which is directly reversible and without side-effects.
In Chapter 1 of this thesis Org 4122, Org 4178, sulfinpyrazone, dipyridamole, $PGE_1$ and $PGI_1$ are studied for these criteria in infusion experiments on dogs without extracorporeal circulation.
In Chapter 2 the results of tests with the drugs which met the requirements most closely are presented. The drugs were tested for their platelet-preserving capacities during CPB in dogs.
In Chapter 3 the main factors which could affect platelets in clinical CPB, such as the type of oxygenator, cardiotomy suction and protamine administration, are studied in order to standardize the clinical protocol for evaluating the selected platelet-inhibiting drugs tested in the animal experiments.
In Chapter 4 the results of tests of the effect of $PGE_1$ on platelet preservation in patients undergoing CPB is presented.

In Chapter 5, the hemodynamic side-effects of $PGE_1$ in clinical CPB are quantitatively evaluated.
In the Epilogue, the hemodynamic and platelet preserving effects of $PGI_2$ in six clinical studies are reviewed.

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CHAPTER 1

# THE EFFECT OF PLATELET FUNCTION INHIBITING DRUGS ON THE PLATELET NUMBER AND FUNCTION ($OD_{max}$) IN DOGS

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## SUMMARY

Impaired hemostasis after cardiopulmonary bypass (CPB) is associated with reduced platelet number and function. Drugs which induce a moderate inhibition of platelet function have failed to preserve the hemostatic capacities of the platelets through CPB. For application during open heart surgery complete inhibition of platelet function with loss of hemostatic capacity would be acceptable as long as the inhibition of platelet function could be immediately reversed after the CPB period. Such an inhibition of platelet function should preserve the platelets during CPB and should result in normal hemostasis thereafter.

In this study the experimental compounds Org 4178 and Org 4122, sulfinpyrazone, dipyridamole, prostaglandin $E_1$ ($PGE_1$) and prostacyclin ($PGI_2$) are tested for their inhibiting effect on platelet function (ADP induced aggregation) and the reversibility of this inhibition in dogs.

Org 4178 and 4122 had a weak and irreversible inhibiting effect on platelet function. Sulfinpyrazone and dipyridamole had a moderate and irreversible inhibiting effect on platelet function. $PGE_1$ and $PGI_2$ gave a strong and reversible inhibition of platelet function.

We conclude that $PGE_1$ and $PGI_2$ are the only drugs that meet the requirements for potential use of platelet preservation during CPB.

## INTRODUCTION

Extracorporeal circulation of blood in cardiopulmonary bypass (CPB) systems causes blood damage. This blood damage results in disturbed hemostasis. Platelet dysfunction and thrombocytopenia are important com-

ponents of the hemostatic disorders in CPB surgery[1]. Platelets adhere to the synthetic surfaces of the extracorporeal circuit which results in aggregation and release reactions. These lead to a decrease in platelet number and function. Pharmacological inhibition of platelet activation during the period of CPB might prevent this self-destructing mechanism and preserve the hemostatic function of the platelets[2,3].

Inhibition of platelet activation during CPB should be sufficiently potent to overcome the strong stimulus of platelet activation by the CPB systems. This inhibition should also be immediately reversible at the end of CPB to restore the hemostatic capacity.

This study was designed to select a platelet function inhibiting drug with an immediate strong effect which is rapid and complete reversible. The drugs studied were: the experimental compounds Org 4178, Org 4122, sulfinpyrazone, dipyridamole, prostaglandin $E_1$ ($PGE_1$) and prostacyclin ($PGI_2$). In this study no extracorporeal circuits were used.

## MATERIALS AND METHODS

Mongrel dogs (15-25 kg BW) were premedicated with 0.5 mg of atropine sulphate, anaesthetized with 20 mg/kg of thiopental sodium, intubated and artificially ventilated (Servo 900B*). Anaesthesia was maintained by ventilating with a mixture of nitrous oxide and oxygen and infusion of 15 mg of piritramide over 5 hours. Pancuroniumbromide (0.08 mg/kg) was given for muscle relaxation and antagonized with 0.5-1 mg of neostigmine sulphate at the end of anaesthesia.

Table 1 lists the dosage of the various platelet function inhibiting drugs, the infusion periods, the administration of heparin and protamine and the number of experiments. Most of the animals were heparinized (200 U/kg) and were given protamine hydrochloride (200 IU/kg) after one or two hours to simulate the situation during cardiopulmonary bypass. This was done because of the important role heparin and protamine could play in platelet kinetics during CPB. As a control, the prostaglandins were also given without heparin and protamine.

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Table 1

| Drug | Dosage ($kg^{-1}$.BW) | Duration of infusion | Heparin | Protamine at ... min. | Number of dogs |
|---|---|---|---|---|---|
| Org 4178 | 3.5 mg | bolus | + | 60 | n = 1 |
| Org 4122 | 10 + 25 mg | 2 x 5 mins | + | 60 | n = 1 |
| Sulfinpyrazone | 12.5 mg | bolus | + | 60 | n = 2 |
| Sulfinpyrazone | 25 mg | bolus | + | 60 | n = 2 |
| Sulfinpyrazone | 50 mg | bolus | + | 60 | n = 5 |
| Dipyridamole | 4 + 4 mg | bolus + 120 mins | + | 120 | n = 1 |
| Dipyridamole | 4 + 4 + 4 mg | 2 x bolus and 120 mins | + | 120 | n = 3 |
| Prostaglandin $E_1$ | 0.2 $\mu g.min^{-1}$ | 60 mins | + | 60 | n = 3 |
| Prostaglandin $E_1$ | 0.2 $\mu g.min^{-1}$ | 60 mins | — | — | n = 3 |
| Tromethamine | | 120 mins | + | 120 | n = 3 |
| Prostacyclin | 0.5-1 $\mu g.min^{-1}$ | 120 mins | + | 120 | n = 3 |
| Prostacyclin | 0.5-1 $\mu g.min^{-1}$ | 120 mins | — | — | n = 3 |

All drugs were administered intravenously. Crystalline Org 4178 and 4122 (Organon, Oss, The Netherlands) were dissolved in saline in a concentration of 10 and 25 mg per ml, respectively.

Sulfinpyrazone (Ciba-Geigy, Arnhem, The Netherlands) was given in a concentration of 100 mg per ml.

Dipyridamole (Boehringer-Ingelheim, Alkmaar, The Netherlands) was given as a bolus injection of 4 mg per kg. In one experiment this injection was followed by an infusion over 120 minutes of 4 mg/kg of dipyridamole dissolved in saline (16 $\mu$g/ml). In three experiments a second bolus injection of 4 mg of dipyridamole per kg was given 30 minutes after the first injection and during the 120-minute infusion of dipyridamole.

Prostaglandin $E_1$ (generously supplied by Unilever Research Laboratories, Vlaardingen, The Netherlands) was dissolved in a 95% ethanol stock solution and frozen at —20°C. For use this solution was diluted in saline (2.4 $\mu$g/ml).

The effect of the tromethamine (THAM) buffer (pH = 8.4), used to dissolve $PGI_2$, was separately tested at an infusion rate of 2 ml/min. THAM was kept at 0°C (Hetofrig*) throughout the infusion period. Prostacyclin sodium salt, 15 $\mu$g/ml (generously supplied by Wellcome Research Laboratories, Beckenham, Kent, England), dissolved in the THAM buffer, has a half life of 5.9 hours at 0°C. The $PGI_2$ infusion rate was decreased when the mean

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arterial blood pressure dropped below 70 mm Hg.
Blood samples were collected from the femoral artery at regular time intervals and from venous puncture on the postoperative days. During infusion of $PGE_1$ samples were also collected from the pulmonary artery. Determined were: hematocrit, hemoglobin, and cell counts for platelets, red- and white blood cells (Coulter ZB-I*). Platelet function was studied with the turbidimetric method[4], using 1 ml platelet rich plasma (PRP) in polystyrene cuvettes placed in an aggregometer (Vitatron UPS**). PRP was prepared by centrifuging 9 ml blood mixed with 1 ml 3.06% sodium citrate at 160 G for 10 minutes. Platelet poor plasma (PPP) was obtained by centrifugation at 1400 G over 10 minutes. The aggregation was induced by the addition of adenosine diphosphate (ADP, Merck B.V., Amsterdam, The Netherlands) in a final concentration of 40 $\mu$M. Aggregation was expressed as the maximal percent increase in light transmission of PRP compared to PPP. Coagulations tests included the bleeding time and fibrinogen. Blood gases were closely monitored (ABL-2 Blood Gas Analyser***) to maintain physiological levels. Arterial and venous blood pressures were monitored on a six-channel recorder (Hewlett-Packard****). Cardiac output was measured by thermodilution, using a Swan-Ganz catheter*****.
Data were processed using a PDP 11/10 computer******. For comparison the individual changes were usually calculated as percentage change from the value obtained after heparinization; when no heparin was employed, changes were calculated from the value obtained before infusion of the drug and in the $PGI_2$ group they were calculated from the value before heparinization. The two tailed Student "t" test was used for statistical evaluation of differences.

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****** Digital Equipment B.V., Utrecht, The Netherlands

## RESULTS

### Org 4178 and Org 4122

Injection of 3.5 mg/kg of Org 4178 had no discernable effect on platelet number or function. However, the dog developed a severe metabolic acidosis and succumbed four hours after the injection.

Platelet function remained unchanged after the injection of 10 mg/kg of Org 4122 for a period of an hour but decreased to 57% of the initial value after protamine was given. At 180 minutes platelet function returned to 66% and a second dose of 25 mg/kg was given. Now a fall in platelet function to 20% occurred followed by a recovery to a level of 75% at 210, 240 and 270 minutes after the first injection. In the following days platelet function returned to the normal range. Platelet numbers were not affected by Org 4122. A metabolic acidosis which developed shortly after the first injection was corrected by frequent administration of sodium bicarbonate solution.

### Sulfinpyrazone

Platelet function decreased abruptly after injection of 12.5, 25 and 50 mg/kg sulfinpyrazone to 79, 56 and 38%, respectively (figure 1). Platelet function returned to normal within 60 minutes after the 12.5 mg dose, but decreased again after administration of protamine to about 55% at 120 minutes. After the 25 mg/kg dose a recovery to about 65% was reached at 30 minutes but platelet function decreased after administration of protamine to about the same level as in the previous group. After the dose of 50 mg/kg of sulfinpyrazone platelet function remained inhibited at a level of about 35% and was slightly affected by protamine administration. At 240 minutes after the injection of the various doses, platelet function in each group reached about the same level of about 35% of initial values. After an initial rise on day 1, a dip was seen on day 2, whereafter function restored to normal.

Platelet counts (figure 2) remained almost unchanged during the first hour after 50 mg/kg of sulfinpyrazone. However, after administration of protamine, platelets counts fell from 96 to 60% ($p < 0.05$), but returned to about 90% at 240 minutes. In this group a dip to 74% occurred on day 2. Platelet counts increased to values between 135-153% in the second week.

The white blood cell (WBC) count was influenced to the same extent by the different dosages of sulfinpyrazone. No change in WBC count occurred during the first hour after the injection, but a leucocytosis developed to

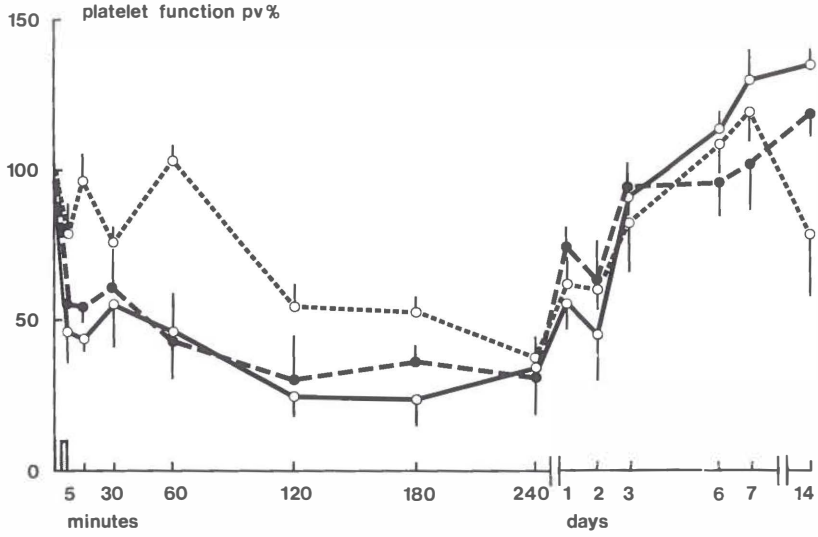


Figure 1. The effect of sulfinpyrazone on platelet function (ADP-induced aggregation). o-----o 12.5 mg/kg; ●——● 25 mg/kg; o——o 50 mg/kg.
In this and subsequent figures, the percentage changes from the pre-infusion values are given (pv %). The points represent means, and the bar, one standard error of the mean. The shaded area represents the period of drug administration.

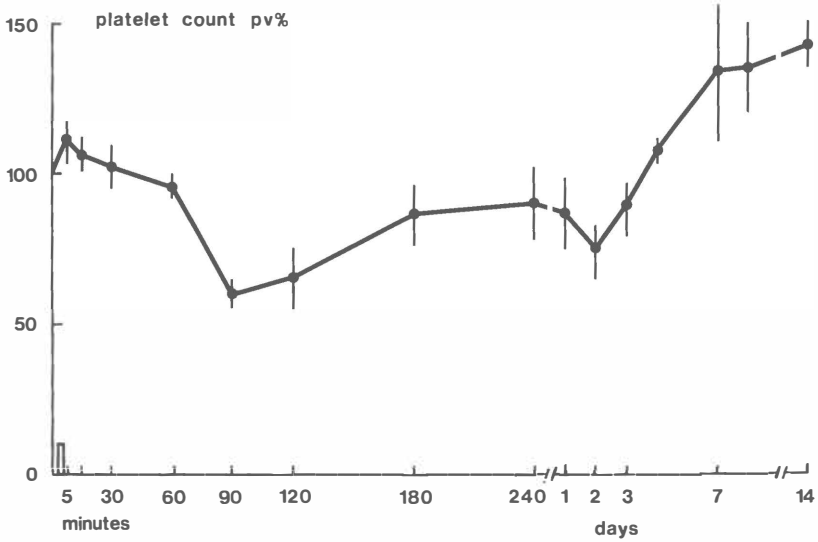


Figure 2. The effect of intravenously administered sulfinpyrazone (50 mg/kg) on platelet count.

values between 170% and 180% after 240 minutes. WBC count had returned to the normal range in all dogs by day 3. Red blood cells, blood pressures, fibrinogen levels and bleeding times were not influenced by sulfinpyrazone.

### Dipyridamole

After an initial dose of 4 mg/kg of dipyridamole, followed by a 2 hour infusion of 4 mg/kg, platelet function decreased to about 60% during the first 30 minutes and recovered to about 75% at 120 minutes. After protamine had been given platelet function decreased to 60% and 38% at 180 and 240 minutes, respectively. Platelet function recovered to about 75% on day 1 and to normal thereafter.

In the group which received a second dose of dipyridamole of 4 mg/kg after 30 minutes of the 120 minutes infusion of dipyridamole, platelet function decreased to 44% at 15 minutes, but increased to 61% at 120 minutes (figure 3); after protamine administration platelet functions of 42% and 49% were measured at 180 and 240 minutes. Platelet function recovered to 56%, 68% and 73% on day 1, 2 and 3 respectively and returned to normal thereafter.

Platelet counts were minimally affected by dipyridamole on the day of experiment, but had decreased to 68% on day 2 and increased to a maximum of 174% in the second week.

White blood cell counts decreased slightly to 87% at 30 minutes and increased subsequently to a level of 250% at 240 minutes. This leukocytosis was followed by a return to the normal range on day 1.

Red blood cells and fibrinogen were not affected by dipyridamole. Administration of dipyridamole caused an abrupt fall in mean arterial blood pressure, from about 150 to 100 mm Hg with a gradual return to values between 120 and 130 mm Hg.

### Prostaglandin $E_1$

During the $PGE_1$ infusion, platelet function (figure 4) decreased in the samples taken from the pulmonary artery to a level of about 40% in the non-heparinized dogs. Platelet function, measured simultaneously in samples taken from the femoral artery, remained in the heparinized and non-heparinized dogs within the normal range. In the non-heparinized dogs to which no protamine was administered either, platelet function, measured in samples taken from the pulmonary artery, returned to normal after the $PGE_1$ infusion was stopped. Platelet function decreased to 62% at 90 minutes and to 37% at 240 minutes in the group in which heparin and at

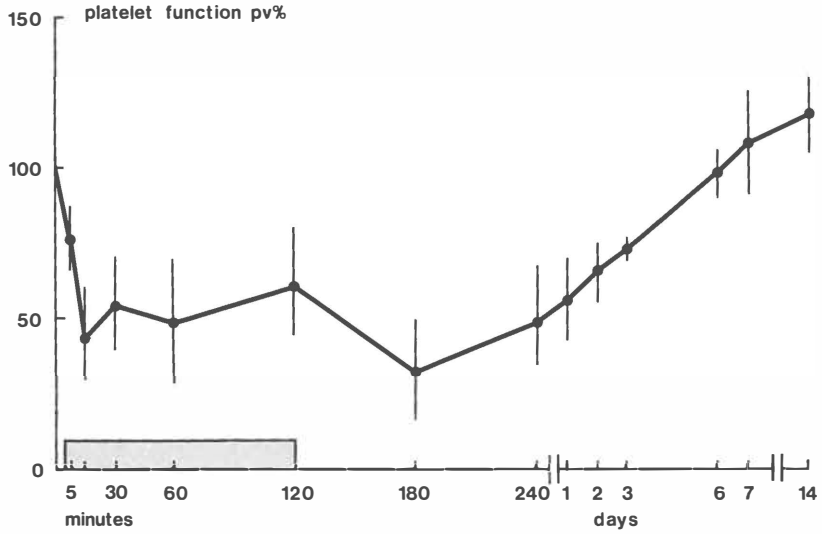


Figure 3. The effect of intravenously administered dipyridamole (4 + 4 + 4 mg/kg) on platelet function (ADP-induced aggregation).

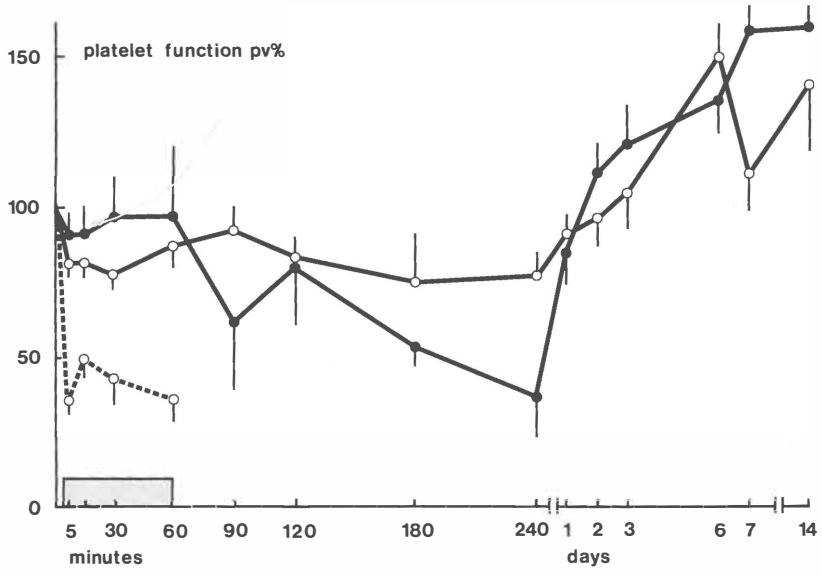


Figure 4. The effect of $PGE_1$ on platelet function (ADP-induced aggregation) in the pre-pulmonary samples o-----o and in the post-pulmonary samples with ●——● and without o——o heparin and protamine administration.

60 minutes protamine were administered. On day 1 a restoration to 84% could be noted in this group, while in the non-heparinized dogs 93% was reached. Platelet function increased in both groups on the following days. Platelet counts (figure 5) were not influenced in the heparinized dogs during $PGE_1$ infusion, whereas in the non-heparinized group a slight but not significant decrease occurred to about 85%. In the heparinized group platelet counts decreased from 106% to 67% ($p < 0.05$) at 90 minutes after protamine was administered, but returned to the normal range in the following period.
The white blood cell (WBC) count remained within the normal range in the non-heparinized $PGE_1$ treated group. In the heparinized $PGE_1$ group WBC counts increased to 167% on day 1 and returned gradually to normal thereafter.
Red blood cells and fibrinogen were not affected in these groups. The systemic blood pressure was only slightly decreased by the $PGE_1$ infusions.

### Prostacyclin ($PGI_2$)

Platelet function decreased after induction of anaesthesia, to about 83% whether or not heparin was given (figure 6). Platelet function remained at this level during the infusion of THAM, but decreased after protamine had been given to 52% at 180 minutes. During the $PGI_2$ infusions platelet function decreased to values between 10% and 32% ($p < 0.05$). After the infusion of $PGI_2$ was stopped and protamine was given, platelet function returned to 49% at 180 minutes and to normal on day 1. When $PGI_2$ was infused without heparin and protamine, platelet function recovered completely after the infusion was stopped and increased slightly during the follow-up period. The platelet count was not influenced by anaesthesia, heparinization, infusion of $PGI_2$ or Tromethamine. However, platelet counts did decrease at 180 minutes, after administration of protamine in the THAM group and the heparinized $PGI_2$ group to 63 and 70% respectively. In all groups an increase in platelet counts started around day three and mean values of between 141% and 212% were reached in the second week. The white blood cell counts increased in these three groups up to 123-145% during the infusion period, reached maximum levels of about 250% on day 1 and returned to normal thereafter.
Red blood cells, fibrinogen and bleeding times were not influenced in these groups. The average systemic blood pressure remained between 125 and 150 mm Hg during the infusion of THAM. Immediately after the start of the

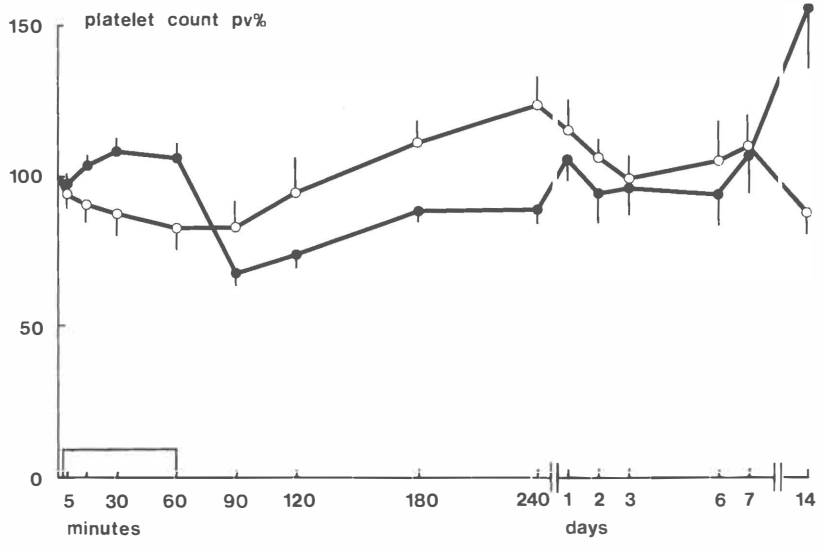


Figure 5. The effect of $PGE_1$ on platelet count in dogs with ●——● and without o——o heparin and subsequent protamine administration.

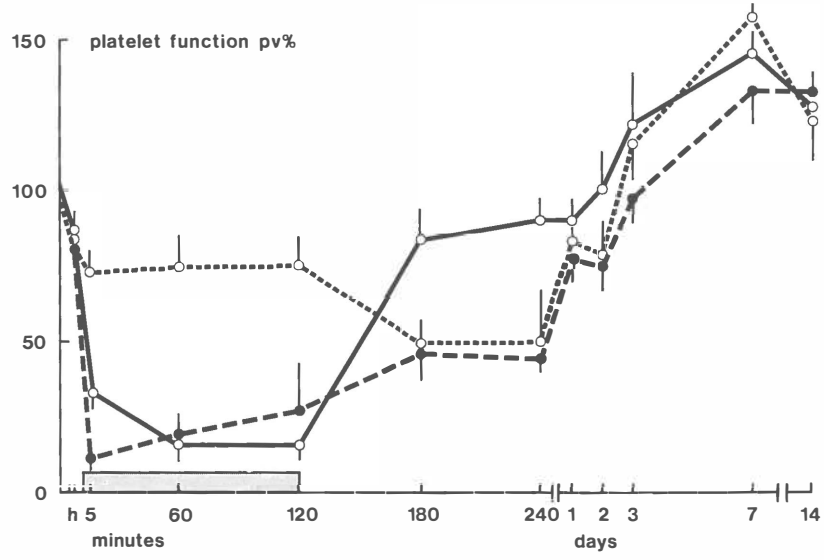


Figure 6. The effect of $PGI_2$ on platelet function (ADP-induced aggregation) in dogs with ●——● and without o——o heparin (h) and protamine administration. o-----o represents the infusion of the THAM buffer solution with heparin and protamine administration.

$PGI_2$ infusions blood pressures dropped but were maintained between 70-75 mm Hg by regulating the infusion rate. After the $PGI_2$ infusion was stopped, the blood pressures returned within 10 minutes to the pre-infusion level. The heart rate increased during $PGI_2$ infusion, while the cardiac output decreased slightly. Pulmonary artery and central venous pressures decreased during the $PGI_2$ infusion.

## DISCUSSION

Treatment with platelet function inhibiting drugs has been proposed to prevent platelet damage during cardiopulmonary bypass (CPB) of blood[2,3]. Experiments with dipyridamole[5,6], sulfinpyrazone[7], $PGE_1$[8,9] and $PGI_2$[10] have been reported.

For application during CPB the effect of the drugs should be short lasting, well controlable by intravenous administration and very potent to overcome the strong platelet activating effect of the CPB systems.

Org 4178 and Org 4122 have been developed as antithrombotic drugs and are only available for laboratory research. In rats and rabbits the platelet inhibition of one single injection lasts between 17 and 24 hours. In our experiments in dogs no effect on platelet function was seen except after administration of protamine. However, an undesirable side-effect on the acid-base balance was observed. These drugs should not be given during clinical CPB.

Sulfinpyrazone is a pyrazole derivative potent in lowering the plasma uric acid level. Its antiplatelet effect is probably due to a reversible competitive inhibition of prostaglandin synthesis[11]. When tested in vitro, sulfinpyrazone inhibits the platelet release reaction, the secondary ADP-induced aggregation and platelet adherence to collagen. Sulfinpyrazone has been reported to preserve platelet counts during membrane oxygenator perfusion in sheep, while apparently normal aggregation was preserved[7].

Dipyridamole belongs to the pyrimido-pyrimidine compounds. It raises the intracellular concentration of cyclic AMP by inhibiting the enzyme phosphodiesterase. Increased levels of cyclic AMP prevent platelet aggregation[12]. In vitro, dipyridamole inhibits both primary and secondary aggregation induced by ADP, epinephrine and collagen, and decreases platelet adhesion to glass bead columns[13]. Dipyridamole also reduces microaggregate formation during in vitro recirculation of canine blood[5]. However, in sheep no increase of platelet number or platelet survival could be detected after membrane oxygenator perfusion[14]. In clinical open heart surgery dipyri-

damole could improve platelet numbers but failed to reduce the post-operative bleeding tendency[6].

In the present study, the platelet inhibiting effect of sulfinpyrazone and dipyridamole is confirmed. This effect is strongly dose related for sulfinpyrazone, but even with the largest dose of 50 mg/kg, platelet function decreased only 50%. Also no strong inhibition of platelet function could be obtained with dipyridamole despite continuous infusion and additional bolus injections. The prolonged inhibition of platelet function can only partly be attributed to the effect of protamine. The inhibition of platelet function by sulfinpyrazone as well as by dipyridamole lasted for several hours, which makes these drugs undesirable for use during CPB.

Prostaglandins are naturally occurring substances, formed from unsaturated fatty acids. $PGE_1$ is formed from the precursor dihomo-gamma-linolenic acid and arises the platelet cAMP level by stimulating adenylcyclase. $PGE_1$ is almost completely metabolized in one single passage through the lung. $PGE_1$ preserved platelet numbers during in vitro recirculation of human blood through an oxygenator[8]. $PGE_1$ was also shown to be able to preserve platelet numbers and function during extracorporeal oxygenation of monkeys[9] and dogs[15].

The present study shows the potent and immediate reversibility of inhibition of platelet function by $PGE_1$ in the pulmonary artery blood samples, while no distinct inhibition was seen in the samples from the systemic circulation. The systemic blood pressures of the dogs were only slightly affected during the infusion of $PGE_1$. If $PGE_1$ is administered during CPB the metabolization of $PGE_1$ by the lung will not take place. In this application a stronger inhibiting effect on platelet function might be expected.

Prostacyclin is generated from arachidonic acid metabolites by the endothelial cells. $PGI_2$ prevents platelet aggregation and adhesion of platelets to the vascular wall. It has an in vivo half-life of 2-3 minutes[16]. $PGI_2$ preserved platelet number and function, decreased consumption of fibrinogen and diminished platelet deposition on arterial filters during CPB in dogs[15]. $PGI_2$ also prevented platelet loss and release of granular contents, while platelet reactivity and subcellular architecture remained preserved during in vitro recirculation of human blood in an oxygenator[17]. In the present study, $PGI_2$ was shown to be a potent and immediately reversible inhibitor of platelet function. During infusion of $PGI_2$ the hypotensive side-effect was more apparent than during infusion of $PGE_1$. This might be explained by the fact that $PGE_1$ is metabolized in the lung.

Heparin and protamine are needed to control the activation of blood coagulation factors during CPB. A platelet aggregating effect has been reported for both drugs[18]. Administration of heparin in man can decrease the platelet release reaction in response to collagen, epinephrine and ADP and prolong the bleeding time. Although this effect of heparin on platelets is reversible, its duration of action is individually different and might exceed two hours. In the present experiments only a weak and transient effect of heparin on the platelets was noted. The effect of protamine on the platelets was consistent. Because protamine is given after bypass to restore hemostasis, this side-effect, which affects hemostasis, is of importance. After the administration of protamine a strong inhibition of platelet function and a reduction of platelet counts was seen in all groups. Prolonged administration of $PGI_2$ might protect the platelets from this undesirable side-effect of protamine. It can be concluded from this study that prostaglandin $E_1$ and prostacyclin are the most suitable drugs to be tested during CPB. Their inhibition of the platelet activation is very potent and reversible immediately after the infusion of these prostaglandins is stopped.

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CHAPTER 2

# PRESERVATION OF PLATELETS WITH PROSTAGLANDIN $E_1$ ($PGE_1$) AND PROSTACYCLIN ($PGI_2$) DURING CARDIOPULMONARY BYPASS (CPB) IN DOGS*

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## SUMMARY

The purpose of this study was to compare the platelet function-inhibiting drugs prostaglandin $E_1$ ($PGE_1$) and prostacyclin ($PGI_2$) for their platelet preserving capacity during cardiopulmonary bypass (CPB). Eighteen dogs underwent CPB by means of a bubble oxygenator for 120 minutes. The dogs were divided into three groups: control dogs ($n = 6$), dogs receiving $PGE_1$ ($n = 6$) and dogs receiving $PGI_2$ ($n = 6$). The prostaglandins were administered as a constant infusion during CPB.

In the control group platelet numbers decreased by 50% and platelet function decreased by 80 % during and after CPB. In the $PGE_1$-treated group, platelet numbers decreased by 32% ($p < 0.02$) and platelet function by 50% ($p < 0.025$) in the same period. In the $PGI_2$-treated group platelet numbers decreased by 28% ($p < 0.05$) and platelet function by about 50% ($p < 0.01$). The average post-bypass bleeding time was more than 15 minutes in the control group, but in both prostaglandin-treated groups the average bleeding times were less than six minutes.

We conclude that both $PGE_1$ and $PGI_2$ help significantly and to the same extent to preserve platelets during CPB in dogs and help to maintain near normal postoperative hemostasis.

* For this chapter the original article has been revised. The part on treatment of patients with $PGE_1$ is left out because this is more extensively described in Chapter 4.

## INTRODUCTION

Cardiopulmonary bypass (CPB) is routinely performed nowadays to sustain patients during cardiac surgery. However, a major problem remaining is that many blood transfusions are required to replace the loss of blood caused by disturbances of the hemostatic mechanism. The changes that frequently lead to disturbed hemostasis are a decrease in platelet number and impaired platelet function[1,2].

During CPB the hemostatic mechanisms are activated by the extracorporeal circuit. Although the clotting cascade is inhibited by heparin during CPB, platelets remain activated. Inhibition of platelet function during CPB would be advantageous and has been shown to preserve platelet numbers and function and to decrease the postoperative bleeding tendency[3,4,5].

In this study a comparison is made of the effectiveness of prostaglandin $E_1$ ($PGE_1$) and prostacyclin ($PGI_2$), in preserving platelets during CPB in dogs.

## MATERIALS AND METHODS

Eighteen mongrel dogs (25-35 kg) were anaesthetized with thiopental sodium (20 mg per kg), intubated and mechanically ventilated. Anaesthesia was maintained with a nitrous oxide/oxygen mixture and 15 mg of piritramide. Pancuronium bromide (0.2 mg per kg) was given for muscle relaxation and antagonized with 0.5 mg of neostigmin sulphate at the end of anaesthesia.

The dogs were given 200 I.U. of heparin per kg. The extracorporeal circuit consisted of polyvinylchloride (PVC) tubing, a non-occlusive roller pump (Dreissen*) and a bubble oxygenator (Temptrol Q-110**). Cannulae were inserted into the femoral and jugular veins and into the femoral artery. The circuit was primed with a mixture of 750 ml of whole blood, drawn on the previous day from healthy dogs and 750 ml of lactated Ringer's solution. The donor blood was stored overnight in ACD (0.14 ml per ml of blood) containing bags and the blood was heparinized (5 I.U. per ml) and recalcified (1 mg per ml) before use. CPB was performed with a flow of 80 to 100 ml per kg per minute, for a duration of 120 minutes.

In the control group ($n = 6$), 2 ml of saline per minute was infused into the venous line. After CPB heparin was neutralized with protamine HCl (1 : 1). In the $PGE_1$-treated group ($n = 6$) the same procedure as in the control

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group was followed, but 20 $\mu$g of $PGE_1$ was added to the priming solution. In addition $PGE_1$ (generously supplied by Organon, Oss, The Netherlands), dissolved in saline, was infused in a dose of 1-2 $\mu$g per kg per minute into the venous line during CPB.

In the $PGI_2$-treated group (n = 6) the same procedure as in the control group was followed, but 0.5-1 $\mu$g of $PGI_2$ (generously supplied by Wellcome Research Laboratories, Beckenham, Kent, United Kingdom) per kg dissolved in a tromethamine (THAM) buffer (pH 8.4 at 0° C) was infused each minute into the venous line during CPB. If necessary the rate of $PGE_1$ or $PGI_2$-infusion was adjusted in order to maintain the mean arterial blood pressure at about 70 mm Hg.

The temperature of the dogs was kept at 35° C by means of heating mattresses and by means of a heat exchanger incorporated into the oxygenator. Penicillin ($4.10^6$ U) and streptomycin (0.5 g) were administered as antibiotic prophylaxis both pre-operatively and on the first three postoperative days.

Blood samples were taken at frequent intervals to determine the platelet count, red and white blood cell counts (Coulter Counter Type ZB-I*), platelet function[6], hematocrit, hemoglobin, plasma hemoglobin[7] and fibrinogen[8]. Platelet function was measured by platelet aggregation and expressed as the maximum percentual change in optical density ($OD_{max}$) of platelet-rich plasma (PRP) compared with platelet poor plasma (PPP), after addition of ADP (Merck B.V., Amsterdam, The Netherlands) to the PRP in a final concentration of 40 $\mu$M. Bleeding time was measured preoperatively and 120 minutes after the end of CPB[9].

Blood gas values were frequently measured (ABL-2 blood gas analyzer**) and maintained at physiological values. Blood pressures were monitored on a six-channel recorder (HP-8800***). All data were processed by a PDP 11/10 minicomputer (DEC****). The two tailed Student's t test was used for statistical evaluation of the differences between the groups.

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** Radiometer A/S, Copenhagen, Denmark
*** Hewlett Packard Benelux, Amstelveen, The Netherlands
**** Digital Equipment BV, Utrecht, The Netherlands

## RESULTS

Platelet count (Fig. 1) decreased in the control group to 66% of the prebypass value (237 ± 72 × $10^9$/l, M ± SD), within five minutes after starting CPB. At the end of CPB platelet count was reduced to 59% of the initial value. In the $PGE_1$-treated group platelet count dropped to 60% of the initial value at five minutes bypass and reached a minimum of 53% at 15 minutes. Thereafter, the platelet count gradually increased to 68% at the end of CPB. In the $PGI_2$-treated group platelet count decreased to 72% of the pre-bypass value at five minutes and remained at this level throughout CPB. During this period platelet counts were consistently but not significantly higher in the $PGI_2$-treated group than in the other two groups. After bypass a further decrease of platelet count was seen in the control group to a minimum value of 45% on day 1. In the $PGE_1$-treated group the platelet count remained at a level of about 70% in the period directly after CPB until the second postoperative day. The highest platelet count of 76% was measured in the $PGI_2$-treated group, 120 minutes after bypass. In the $PGI_2$-treated group the platelet count reached a minimum value of 52% on the second postoperative day. In all groups platelet counts returned to normal values after the second

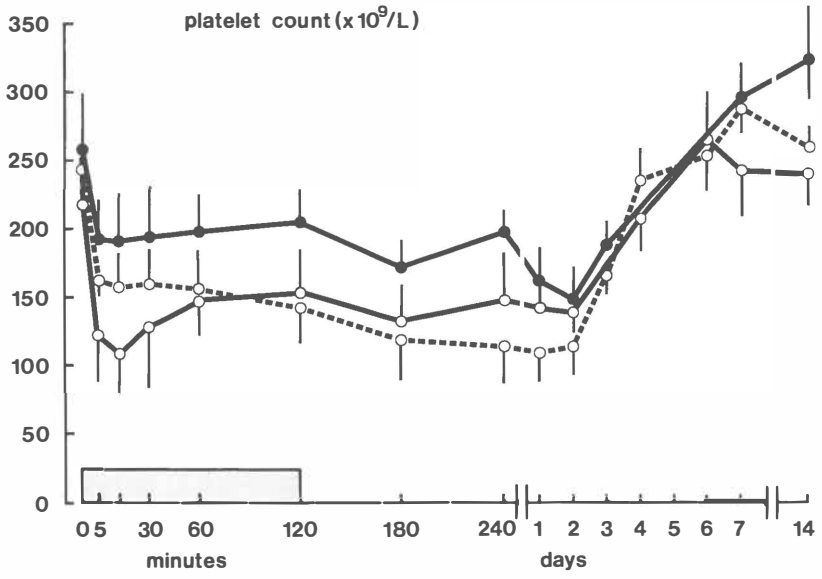


Figure 1. Platelet count during and after CPB in control dogs: o-----o; in $PGE_1$-treated dogs: o——o and in $PGI_2$-treated dogs: ●——●. The points and bars represent means and one standard error of the mean. Shaded area represents period of CPB.

post-operative day. Significant differences in platelet counts in the post-bypass period were established between the control group and $PGE_1$-treated group at 240 minutes ($p < 0.02$), day 1 ($p < 0.05$) and day 2 ($p < 0.05$) and between the control group and $PGI_2$-treated groups at 240 minutes ($p < 0.05$).

Platelet function (initial value: $46 \pm 14\%$) decreased in the control group to 25% at five minutes and to 16% at the end of CPB (Fig. 2). In the $PGE_1$-treated group platelet function decreased to 9% at five minutes and remained inhibited (10%) until the end of CPB. In the $PGI_2$-treated group platelet function decreased to 13% at five minutes and remained at this level for thirty minutes. Thereafter platelet function gradually increased to 27% at the end of CPB. The inhibition of platelet function by both prostaglandins was most obvious during the first thirty minutes of bypass, but only statistically significant for $PGE_1$ at five minutes (Table 1). During the postbypass period, platelet function decreased further to a minimum of 9% in the control group. However, in both prostaglandin treated groups platelet function increased significantly in this period to about 25%. Platelet function was in all groups higher on the second postoperative day and returned thereafter to initial values.

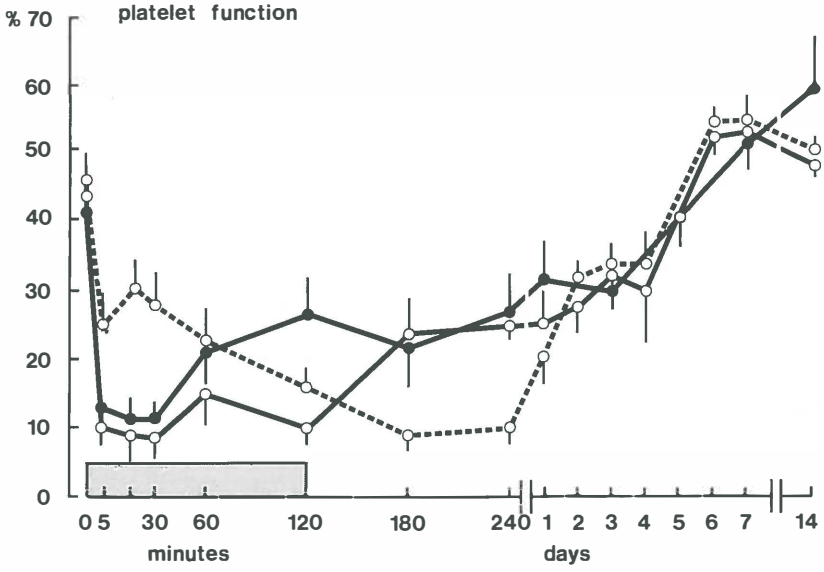


Figure 2: Platelet function ($OD_{max}$) during and after CPB in control dogs: o-----o; in $PGE_1$-treated dogs: o——o and in $PGI_2$-treated dogs: ●——●.

Table 1. Upper limits of p values of platelet function in the control group compared to the $PGE_1$ and $PGI_2$ treated groups, at various times after beginning of CPB. N.S.: not significant.

| minutes | $PGE_1$ | $PGI_2$ |
|---|---|---|
| 5 | 0.02 | N.S. |
| 120 | 0.05 | N.S. |
| 180 | 0.025 | 0.02 |
| 240 | 0.025 | 0.01 |

The initial bleeding time was less than 3 minutes. The average bleeding time in the control group was over 15 minutes, 120 minutes after CPB, while in the prostaglandin treated groups the average bleeding time was less than 6 minutes.

Plasma fibrinogen levels (initial value: $2.3 \pm 0.6$ g/l) decreased equally in all groups, reached a minimum of about 50% of the preoperative values at the end of CPB and increased again to about 70%, 120 minutes after CPB. Maximal plasma fibrinogen levels of about 210% were measured on the second and third postoperative day. On day seven all values had returned to the normal range.

No significant differences in plasma hemoglobin levels were detected among the groups. Pre-bypass levels of $5 \pm 1$ mg% increased in all groups to about 40 mg% at two hours of bypass and returned to normal in all groups on the first postoperative day.

White blood cell count (initial value: $7.1 \pm 1.6 \times 10^9$/l) was 96% after 15 minutes of CPB in the control group, after which a leucocytosis developed to 388% on the first postoperative day. In the $PGE_1$-treated group a minimum of 54% was reached at 15 minutes bypass, after which the white blood cell count increased to 279%, 120 minutes after CPB and reached a maximum of 307% on the first postoperative day. In the $PGI_2$-treated group white blood cell count did not differ from the initial value during the first 60 minutes of bypass. Then white blood cell count increased to 403% at 120 minutes after the end of CPB. White blood cell count gradually returned to the initial value during the first postoperative week. Throughout the entire duration of this investigation no significant differences of white blood cell counts could be detected among the groups. None of the dogs developed clinical symptoms of infection.

## DISCUSSION

To prevent platelet damage during extracorporeal circulation of blood, treatment with platelet function inhibiting drugs has been proposed[10]. Results in animal experiments have been reported for dipyridamole[3 10], sulfinpyrazone[11], cyproheptadin[12], $PGE_1$[4 5] and $PGI_2$[13-15]. The important characteristics of $PGE_1$ and $PGI_2$ is their powerful and transient inhibition of platelet function. Two thirds of $PGE_1$ are metabolized during each passage through the lung, and $PGI_2$ has an in vivo half life of 2-3 minutes. This is in contrast to the irreversible platelet function inhibiting effect of dipyridamole, sulfinpyrazone and cyproheptadin. $PGI_2$ was discovered by Vane and co-workers in 1976 and appeared to be 30 times more potent than $PGE_1$ in its inhibition of platelet function[16]. $PGI_2$ is generated from endoperoxides in the endothelial cells and prevents adhesion of platelets to the healthy vascular wall[17]. Recently it was shown that synthetic $PGI_2$ prevents platelets from sticking to the artificial surfaces of extracorporeal circuits[13]. When $PGI_2$ was used during hemodialysis in dogs thrombocytopenia and platelet microembolization were reduced and no heparin was needed. Improved preservation of platelet count and function, a decreased consumption of fibrinogen and a diminished platelet deposition on arterial filters was reported during CPB in dogs[14]. During in vitro recirculation of heparinized human blood through a membrane oxygenator, $PGI_2$ prevented the loss and release of granule contents of the platelets, while platelet reactivity and subcellular architecture were maintained[15].

In our study administration of $PGE_1$ induced an acute and strong inhibition of platelet function during CPB. This inhibition could be maintained throughout this period and resulted in a statistically significant improvement in platelet function after CPB. The laboratory test of platelet function was performed on platelets in plasma which still contained $PGE_1$. Addonizio et al. have shown that when platelets were separated from their $PGE_1$ containing plasma by gel filtration, platelet function is also better maintained during CPB[4].

The initial fall in platelet count is partly due to hemodilution and therefore not prevented by $PGE_1$. However, the preserving effect was evident because of significantly improved platelet counts in the post-bypass period. The better preserved platelet number and function in the $PGE_1$-treated group correlate with improved bleeding times.

Both $PGI_2$-administration and $PGE_1$-administration induced equally strong and direct inhibitions of platelet function; however, in the $PGI_2$-treated

group during the second hour of CPB, platelet function recovered and became even higher than that in the control group. This unexpected early return of platelet function, also described by Longmore[14], is unexplained but might be due to spontaneous breakdown of the unstable $PGI_2$ in the infusion bottle or in the blood samples. Nevertheless, this treatment resulted in a better preserved platelet function than in the control group.

$PGI_2$-administration helped to preserve platelet numbers during CPB, but a slight decrease could be seen after the administration of protamine. Continuation of $PGI_2$-infusion during the protamine-administration might prevent this adverse effect of protamine on the platelet count[18 19]. The decrease in platelet count in the $PGI_2$-treated group on the first postoperative day indicates that the platelets have been affected by CPB and are prematurely eliminated; thus the protective effect of $PGI_2$ is only partial. However, of main importance is the fact that platelet number and function were better maintained in the early postoperative period, and that consequently bleeding times remained normal.

$PGE_1$ and $PGI_2$ did not decrease red or white blood cell counts. Fibrinogen concentration decreased equally in all groups, despite heparinization. This indicates that during $PGI_2$ treatment fibrinogen is also consumed. Thus $PGI_2$ treatment does not prevent activation of the clotting cascade. Therefore it is not to be expected that $PGI_2$ treatment can replace heparinization during CPB[14].

This study concludes that $PGE_1$ and $PGI_2$ have a similar beneficial capacity to preserve platelet number and function during CPB in dogs. However, it has yet to be determined whether such beneficial effects will reduce the platelet related postoperative bleeding tendency after clinical CPB.

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CHAPTER 3

# CLINICAL STUDY OF BLOOD TRAUMA DURING PERFUSION WITH MEMBRANE AND BUBBLE OXYGENATORS

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## SUMMARY

This clinical study was designed to show differences in hemostasis after perfusion with membrane (MO) or bubble (BO) oxygenators in cardiopulmonary bypass. These differences, however, may be obscured by other damaging factors, such as cardiotomy suction, various perfusion durations, or different types of operations. Therefore, only patients undergoing coronary artery bypass grafting were studied. During such operations, rather equal perfusion durations can be expected and mainly apex vent suction is used. Data on postoperative blood loss and transfusions were collected from two groups: one comprising 49 patients perfused with the Travenol TMO membrane oxygenator (MO group) and the other composed of 25 patients with the Polystan VT 5000 bubble oxygenator (BO group). In 10 patients of each group, blood samples were taken at frequent intervals for blood cell counts, for the determination of platelet function, hematocrit, hemoglobin, and plasma hemoglobin, for several coagulation tests, and for the assessment of kidney and liver function.

Significant differences between the two groups were found for postoperative blood loss and transfusions, both higher in the BO group. Platelet function was better maintained in the MO group until the moment of releasing the aortic cross-clamp, after which it decreased concurrently with a doubling of the amount of suction. After protamine administration, an additional drop of platelet function occurred and there was virtually no platelet function left in either groups; however, it recovered 90 minutes after bypass, mainly in the MO group. This study shows that hemostasis is better preserved with MO perfusion. Still further improvements may be achieved by preventing the damage of suction and by a correct protamine dose.

## INTRODUCTION

Despite careful hemostasis, most cardiopulmonary bypass procedures require transfusions of whole blood and frequently additional blood fractions to control the associated coagulation disorders encountered. The extensive literature on this subject has implicated several causative factors: the type of oxygenator,[1,2] the anticoagulation treatment, and the use of cardiotomy suction[3-8]. None of the clinical reports, however, has evaluated the interaction of all three factors. Such a study must involve a group of comparable patients undergoing the same type of operation[1,9] and comparable perfusion times, cardiotomy suction techniques, and anticoagulant procedures.

This investigation concerns the hematological effects observed in a group of patients undergoing coronary artery bypass grafting during membrane oxygenerator (MO) or bubble oxygenerator (BO) perfusion. Data were obtained to include the effects of cardiotomy suction and protamine termination of anticoagulant effects.

### Patients and methods

A prospective study was conducted on 74 adult patients requiring cardiopulmonary bypass for coronary artery bypass grafting. Perfusion times were comparable and did not exceed 170 minutes. Table 1 shows the distribution of the two groups as to type of oxygenator, age, weight, perfusion time, blood flow rate and lowest temperature. Ten representative patients from each group were selected for the hematologic studies.

Table 1. Patients and perfusion data*

| | MO | BO |
|---|---|---|
| Total No. of patients | 49 | 25 |
| Weight (kg) | 78 ± 8 | 75 ± 8 |
| Age (yr) | 54 ± 6 | 52 ± 8 |
| Perfusion time (min) | 117 ± 27 | 104 ± 29 |
| Blood flow rate (L/min) | 4.5 ± 1.4 | 5.1 ± 0.8 |
| Hypothermia (°C) | 28.8 ± 0.5 | 27.4 ± 0.4 |

* Mean and standard deviation (M ± SD) of perfusion data of patients undergoing coronary artery bypass grafting with a membrane oxygenator (MO) or a bubble oxygenator (BO).

For premedication, nitrazepam (5 mg) and butobarbiton (100 mg) were given orally on the evening before operation and diazepam (15 mg) on the morning of the day of operation.

Anaesthesia was induced with etomidate (0.3 mg/kg, Janssen, Belgium) and maintained with a nitrous oxide/oxygen mixture ($FIo_2$ 0.5). Pancuronium bromide (0.1 mg/kg) was used for muscle relaxation and piritramide (0.75 mg/kg) for analgesia. Droperidol (0.25 mg/kg) was added to achieve neurolept-anaesthesia. Additional doses of these drugs were repeated when necessary.

All patients were ventilated (Servo Ventilator Model 900B and Servo carbon dioxide analyzer Model 930, Siemens-Elema, Division of Elema-Schönander, Inc., Solna, Sweden). Arterial blood samples were taken at intervals to check the adequacy of ventilation as well as to perform the hematologic studies. Heparin (300 U/kg, Leo Pharmaceuticals, Emmen, The Netherlands) was given after the thoracotomy and readministered hourly (100 U/kg). The effect of heparin was reversed within 30 minutes after bypass with protamine hydrochloride (600 U/kg, Hoffmann-La Roche B.V., Midrecht, The Netherlands). In a separate group of patients ($n = 10$) protamine administration was postponed until a blood sample for platelet function study was taken 30 minutes after the end of bypass.

For cardiopulmonary support, the Travenol total bypass membrane oxygenator with polypropylene membrane (TMO Model 5M1430, Travenol Laboratories, Inc., Deerfield, Ill.) or the Polystan Venotherm 5000 bubble oxygenator (VT, Polystan, Copenhagen, Denmark) was used. In all groups one roller pump (Dreissen Modul pump, Hellevoetsluis, The Netherlands) was used for the arterial blood return, one for suction for left ventricle decompression, and two pumps for coronary suction. In the TMO group an extra roller pump was required for the venous return line to the oxygenator. Polyvinyl chloride tubing was used in all cases except for the tubing in the roller pump, which was silicone rubber. The TMO and VT 5000 oxygenator systems, including the tubing, were primed with Ringer's lactate solution, 2000 and 1350 ml respectively, to which human serum albumin (20%), 500 and 357 ml respectively, was added. During bypass, the blood obtained from coronary suction and suction applied for left ventricular decompression was returned to the oxygenator via a cardiotomy reservoir, incorporating a 27 $\mu$ filter (Model Q-220F, Bentley Laboratories, Inc., Irvine, Calif.). A flow transducer, connected to a flowmeter integrator unit (Model 376/393, Nycotron, Drammen, Norway), was placed in the outlet line of the cardio-

tomy reservoir to calculate the total amount of aspirated blood. No extra microemboli filters were used.

Moderate hypothermia (28° to 30°C) was used routinely. The flow rates were 3 L/m² at normothermia or 2.4 L/m² at 28° C with an eventual reduction when the venous oxygen saturation in the venous return line (In Vivo Haemoreflectometer, Schwarzer, Munich, West Germany) was higher than 0.75. After aortic cross-clamping, cardioplegia was achieved with the Bretschneider solution (20 ml/kg) at 4° C.[10] The Bretschneider solution was aspirated into a separate reservoir (Polystan, HL-287/DFA) and centrifuged to prepare red cell concentrate to be returned to the patient. The hematocrit value was kept between 20% and 25%. At the termination of extracorporeal circulation, the patient was rewarmed to 37° C (nasopharyngeal temperature).

Blood loss was measured from chest tube drainage starting in the operating room from the moment of protamine administration until 8:00 A.M. on the

Table 2. Blood transfusion requirements (units)*

| | MO (n = 49) | BO (n = 25) | t Value | p Value |
|---|---|---|---|---|
| Whole blood | | | | |
| Mean ± SEM | 3.8 ± 0.4 | 5.4 ± 0.8 | —1.91 | <0.05 |
| Range | 0 — 7 | 0 — 17 | | |
| Packed cells | | | | |
| Mean ± SEM | 1.0 ± 0.2 | 1.3 ± 0.3 | —1.12 | NS |
| Range | 0 — 4 | 0 — 4 | | |
| Platelet concentrate | | | | |
| Mean ± SEM | 0.4 ± 0.3 | 3.6 ± 1.4 | —3.35 | <0.001 |
| Range | 0 — 6 | 0 — 18 | | |
| Cryoprecipitate | | | | |
| Mean ± SEM | 0.4 ± 0.3 | 3.6 ± 1.6 | —2.68 | <0.01 |
| Range | 0 — 6 | 0 — 32 | | |
| Fresh-frozen plasma | | | | |
| Mean ± SEM | 0.5 ± 0.2 | 3.2 ± 1.5 | —2.54 | <0.01 |
| Range | 0 — 6 | 0 — 32 | | |

* The amount of whole blood and blood products in patients undergoing coronary artery bypass grafting using a membrane oxygenator (MO) or a bubble oxygenator (BO). Values in this and in subsequent tables are given as mean and one standard error of the mean.

first postoperative day in the intensive care unit. Other blood losses, e.g., in sponges, were not determined.

Transfusions of whole blood or packed cells were given to both groups when hemoglobin values dropped below 100 gm/L. Platelet concentrate, cryoprecipitate, and fresh-frozen plasma were infused when diffuse bleeding persisted.

Blood samples were studied at frequent intervals up to the tenth postoperative day for erythrocyte, leukocyte, and platelet counts (Coulter Electronics, Inc., Hialeah, Fla.), hematocrit, hemoglobin, plasma hemoglobin,[11] fibrinogen,[12] fibrinogen degradation products (Thrombo-Wellcotest, Wellcome Reagents Ltd., Beckenham, England), and antithrombin III (Coatest, Kabi Diagnostica, Stockholm, Sweden). Platelet function was expressed by the maximum percentage change in optical density ($OD_{max}$) of platelet-rich plasma compared to platelet-poor plasma, after addition of adenosine diphosphate in a final concentration of 0.83 $\mu$g/ml to the platelet-rich plasma.[13]

Kidney function was studied by measuring blood urea nitrogen (BUN) and serum creatinine. Changes in liver function were established by measuring alkaline phosphatase, lactic dehydrogenase, glutamic and pyruvic transaminases, and total and direct bilirubin (SMA-C, Technicon Instruments Corp., Tarrytown, N.Y.).

Data concerning blood requirements were processed by a DEC 10 computer and the hematological data by a DEC-PDP 11/10 minicomputer. For statistical analysis of differences between the groups, the Student's t test or Mann-Whitney test were used. Values of $p > 0.05$ were considered to be not significant (NS).

## Results

The amount of whole blood and blood fractions transfused for both groups of patients are given in Table 2. In the patients subjected to perfusion with a BO, significantly more whole blood ($p < 0.05$), platelet concentrate ($p < 0.001$), cryoprecipitate ($p < 0.01$), and fresh-frozen plasma ($p < 0.01$) were transfused as compared to the same category of patients perfused with an MO. Also blood loss (mean $\pm$ SEM) in the BO group ($2.0 \pm 0.1$ L) was significantly ($p < 0.01$) higher than in the MO group ($1.3 \pm 0.1$ L).

Differences in hematologic parameters were evaluated in 10 patients of each group.

Plasma hemoglobin levels (normal range 0 to 5 mg/100 ml, Fig. 1) increased equally in the BO and MO groups during perfusion and reached levels of 39 ± 5 mg/100 ml and 34 ± 6 mg/100 ml respectively, at 60 minutes of bypass. Plasma hemoglobin further increased in the BO group to 44 ± 4 mg/100 ml, whereas in the MO group a slight decrease to 26 ± 4 mg/100 ml at 90 minutes was followed by a rise to 37 ± 5 mg/100 ml at the end of bypass. Within 3 days normal values were reached again in both groups. Plasma hemoglobin levels were consistently higher in the BO group, but the differences were significant ($p < 0.05$) only at 90 minutes of bypass and 30 and 60 minutes after bypass.

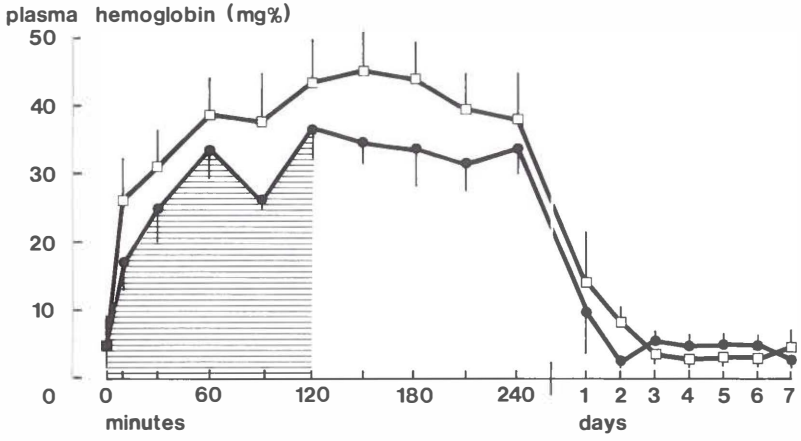


Fig. 1. Plasma hemoglobin. The effect of membrane oxygenator (MO) and bubble oxygenator (BO) perfusion on the plasma hemoglobin values. The points represent means and the error bar represents one standard error of mean in this and in subsequent figures. ●——● The MO-perfused patient group (N = 10). □——□ The BO-perfused patient group (N = 10).

The changes in hematocrit, hemoglobin, and red cell and white blood cell counts are given in Table 3. No significant differences occurred between the groups. The severe drop of the values in the very beginning of the perfusion is related to the type of hemodilution employed, which was compensated by the seventh postoperative day.

Table 3. Changes in red cell and white cell parameters in patients undergoing coronary artery bypass grafting using a membrane (MO) or a bubble oxygenator (BO).

| | Group | Pre-CPB | During CPB (5 min) | End CPB | Post-CPB (2 hr) | Day 7 |
|---|---|---|---|---|---|---|
| Hematocrit | MO | 0.36 ± 0.01 | 0.23 ± 0.01 | 0.22 ± 0.01 | 0.30 ± 0.03 | 0.37 ± 0.01 |
| (42-52%) | BO | 0.38 ± 0.01 | 0.24 ± 0.01 | 0.23 ± 0.01 | 0.29 ± 0.01 | 0.38 ± 0.02 |
| Hemoglobin | MO | 123 ± 4 | 80 ± 5 | 73 ± 3 | 97 ± 8 | 126 ± 6 |
| (140-170 gm/L) | BO | 127 ± 3 | 83 ± 2 | 78 ± 2 | 98 ± 4 | 129 ± 6 |
| RBC count | MO | 3.8 ± 0.1 | 2.6 ± 0.2 | 2.4 ± 0.1 | 3.2 ± 0.2 | 3.9 ± 0.1 |
| (4.6-6.2.$10^{12}$/L) | BO | 4.0 ± 0.1 | 2.6 ± 0.1 | 2.4 ± 0.1 | 3.0 ± 0.1 | 4.0 ± 0.2 |
| WBC count | MO | 5.9 ± 0.5 | 4.0 ± 0.5 | 6.1 ± 1.0 | 6.6 ± 0.9 | 10.0 ± 1.1 |
| (4-11.$10^{9}$/L) | BO | 6.1 ± 0.8 | 4.2 ± 0.7 | 5.2 ± 0.5 | 5.1 ± 0.8 | 9.6 ± 0.7 |

Legend: CPB, Cardiopulmonary bypass. RBC, Red blood cell. WBC, White blood cell.

The platelet count before anaesthesia(Fig.2,mean±SEM) averaged 202 ± 23 and 213 ± 22.$10^9$/L in the MO and BO groups and decreased about 25%, to 169 ± 18 and 158 ± 14.$10^9$/L, during the prebypass period. After 5 minutes of bypass, platelet count dropped in both groups to about 50% of the preoperative values (111 ± 9 and 107 ± 9.$10^9$/L, respectively). After 30 minutes of bypass, platelet numbers improved in the MO group, but in the BO group a gradual further decrease was observed. Platelet counts stabilized after bypass in the MO group, whereas the lower counts in the BO group tended to improve. In this latter group a slight decrease in platelet counts from 81 ± 10 to 67 ± 10.$10^9$/L was seen on the second postoperative day, before a rapid increase, parallel with the MO group, occurred. Normal values were reached around day 5, and slight thrombocytosis to values of 351 ± 50 and 448 ± 25.$10^9$/L, respectively, developed on day 10. Although platelet counts were consistently higher in the MO group during the whole course, significant differences ($p < 0.05$) could be noted only at 90 minutes of bypass.

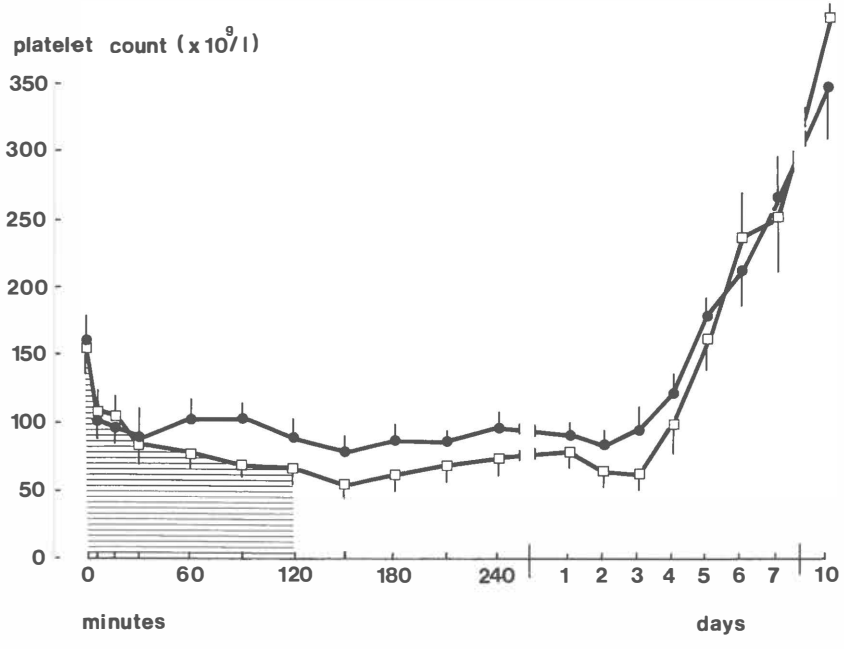


Fig. 2. Platelet count. The effect of membrane oxygenator (MO) and bubble oxygenator (BO) perfusion on the platelet counts. ●——● The MO-perfused patient group (N = 10). □——□ The BO-perfused patient group (N = 10).

The values of the platelet function ($OD_{max}$ mean ± SEM, Fig. 3) averaged 66% ± 9% and 58% ± 7% in the MO and BO groups shortly before bypass. At 5 minutes of bypass, platelet function dropped to 42% ± 6% in the MO group and to 23% ± 3% in the BO group. Platelet function remained low during the whole bypass period in the BO group but improved to subnormal levels (50% to 60%) in the MO group. However, in the MO group, a fall in platelet function from 56% ± 10% to 37% ± 7% occurred after 90 minutes of bypass, coinciding with the second increase in plasma hemoglobin in this group. In this period the aortic cross-clamp is released; blood passing the apex vent and suction from the pericardial space are increasing considerably. In 16 cases the flow through the cardiotomy reservoir was measured and was found to have increased from 206 ml/min during cross-clamping to 526 ml/min after release of the aortic cross-clamp. The average total amount of blood passing through the cardiotomy reservoir during the whole bypass period was 34 L, with a range from 2 to 65 L. Thirty minutes after bypass, when protamine had been given to reverse the heparin effect, another drop in platelet function was observed; the aggregation capacity of the platelets in the early postoperative period was virtually eliminated (7% ± 3%). Consequently, after protamine administration, no differences in platelet function between the groups could be noted up to 90 minutes after bypass. Thereafter, platelet function recovered, but mainly in the MO group. In this group

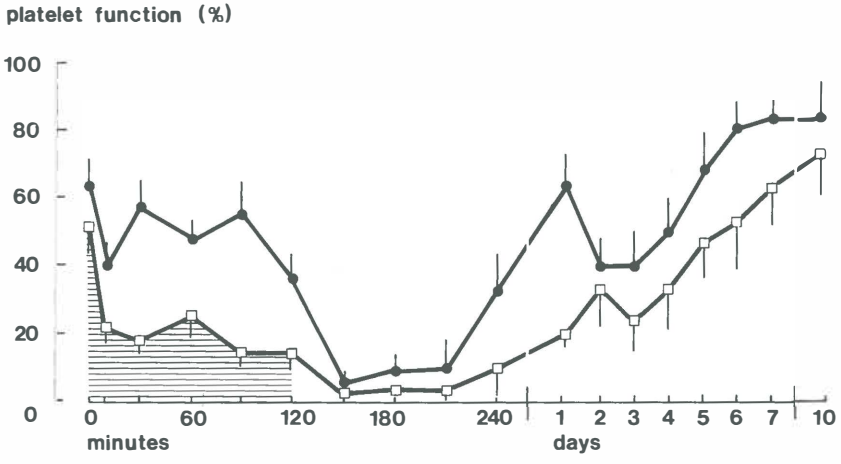


Fig. 3. Platelet function. The effect of membrane oxygenator (MO) and bubble oxygenator (BO) perfusion on the platelet function ($OD_{max}$). ●—● The MO-perfused patient group (N = 10). □——□ The BO-perfused patient group (N = 10).

platelet function returned to the normal range (65% ± 9%) on the first postoperative day, whereas function remained low (19% ± 2%) in the BO group. In both groups function showed a fall on day 2 or 3 before permanent restoration toward normal function started.

The decrease in platelet function in relation to protamine administration is illustrated in Fig. 4. In a separate group of 10 patients undergoing long-term perfusion during coronary bypass grafting (159 ± 28 min) with an MO, protamine administration was postponed until after the 30 minute post-bypass blood sample for platelet function study was taken. In this group of patients, platelet function 30 minutes after bypass remained at the same level as during bypass. The same drop in platelet function could be reproduced after the postponed dose of protamine was given.

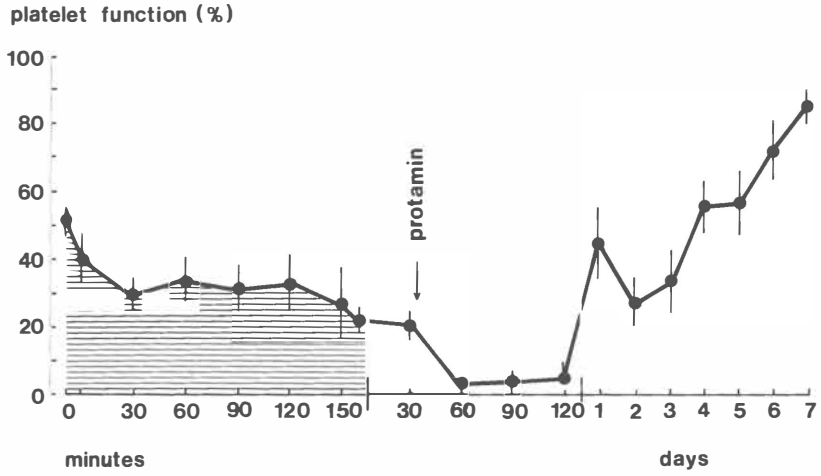


Fig. 4. Platelet function. The effect of postponed protamine administration on the platelet function ($OD_{max}$). Protamine was administered 30 minutes after the end of bypass (N = 10).

The changes in fibrinogen, fibrinogen degradation products and antithrombin III are given in Table 4. All the changes were similar in both groups and no statistical differences were noted. Fibrinogen decreased concomitantly with the hematocrit value, in relation to hemodilution in the first hours after bypass, and became normal on the first postoperative day. On day 7 postoperatively, increased values were measured. Fibrinogen degradation products increased only slightly until the second postoperative day but were clearly elevated on day seven postoperatively. Antithrombin III

was slightly decreased until the second postoperative day and was normal on day 7 postoperatively.

Table 4. Changes in coagulation parameters in patients undergoing coronary artery bypass grafting using a membrane (MO) or a bubble oxygenator (BO).

| | Group | Pre-CPB | Post-CPB (2 hr) | Day 1 | Day 7 |
|---|---|---|---|---|---|
| Fibrinogen (2-4 gm/L) | MO | 4.1 ± 0.5 | 2.2 ± 0.4 | 3.9 ± 0.4 | 6.3 ± 0.6 |
| | BO | 3.2 ± 0.2 | 2.3 ± 0.3 | 3.4 ± 0.2 | 6.5 ± 0.3 |
| FDP (0-10 mg/L) | MO | 10 ± 0 | 10 ± 1 | 10 ± 0 | 22 ± 3 |
| | BO | 10 ± 0 | 13 ± 2 | 12 ± 1 | 20 ± 4 |
| AT III (95%-120%) | MO | — | 78 ± 10 | 60 ± 8 | 111 ± 4 |
| | BO | — | 74 ± 6 | 74 ± 6 | 112 ± 2 |

Legend: CPB, Cardiopulmonary bypass. FDP, Fibrinogen degradation products. AT III, Antithrombin III.

The changes in BUN and serum creatinine are given in Table 5. Although BUN and creatinine levels increased slightly in the BO group, the values generally remained within the normal range 2 hours after perfusion. BUN levels were elevated in both groups on day 7, and the creatinine level was elevated in the MO group. However, no statistical differences were observed between the groups at any time.

Table 5. Changes in renal function in patients undergoing coronary artery bypass grafting using a membrane (MO) or a bubble oxygenator (BO).

| | Group | Pre-CPB | Post-CPB (2 hr) | Day 1 | Day 7 |
|---|---|---|---|---|---|
| Blood urea nitrogen | MO | 5.6 ± 1.0 | 5.3 ± 0.1 | 5.2 ± 0.3 | 10.5 ± 1.6 |
| (3.3-6.7 mmol/L) | BO | 5.9 ± 0.6 | 7.3 ± 1.3 | 5.3 ± 0.6 | 10.2 ± 1.2 |
| Serum creatinine | MO | 83 ± 13 | 83 ± 15 | 92 ± 5 | 123 ± 43 |
| (62-106 μmol/L) | BO | 84 ± 6 | 123 ± 26 | 89 ± 8 | 96 ± 5 |

Legend: CPB, Cardiopulmonary bypass.

The changes in alkaline phosphatase, lactic dehydrogenase, glutamic and pyruvic transaminase, and bilirubin values are given in Table 6. The changes in both groups were about the same and no statistical differences occurred. Alkaline phosphatase was only slightly elevated in the MO group 7 days after operation. Lactic dehydrogenase was elevated in both groups after perfusion and remained so until day 7 postoperatively. Glutamic oxaloacetic transaminase remained essentially normal throughout the whole observation period, whereas glutamic pyruvic transaminase was elevated in both groups on day 7 postoperatively. Total bilirubin increased after perfusion but remained in the normal range throughout the observation period, except on day 1 in the MO group. Direct bilirubin value was slightly elevated in the MO group after perfusion and remained so until day 7 postoperatively.

Table 6. Changes in liver function in patients undergoing coronary artery bypass grafting using a membrane (MO) or a bubble oxygenator (BO).

| | Group | Pre-CPB | Post-CPB (2 hr) | Day 1 | Day 7 |
|---|---|---|---|---|---|
| Alkaline phosphatase | MO | 71 ± 4 | 40 ± 8 | 51 ± 7 | 152 ± 46 |
| (13-120 U/L) | BO | 62 ± 13 | 42 ± 8 | 43 ± 7 | 98 ± 14 |
| Lactic dehydrogenase | MO | 172 ± 19 | 326 ± 9 | 346 ± 19 | 333 ± 13 |
| (114-235 U/L) | BO | 211 ± 2 | 459 ± 47 | 456 ± 53 | 366 ± 36 |
| Glutamic oxaloacetic transaminase | MO | 23 ± 2 | 53 ± 25 | 47 ± 7 | 40 ± 4 |
| (0-40 U/L) | BO | 18 ± 2 | 40 ± 6 | 45 ± 7 | 36 ± 3 |
| Glutamic pyruvic transaminase (0-30 U/L) | MO | 21 ± 5 | 17 ± 1 | 18 ± 2 | 53 ± 9 |
| | BO | 23 ± 8 | 14 ± 2 | 20 ± 3 | 60 ± 10 |
| Total bilirubin | MO | 7 ± 1 | 21 ± 3 | 36 ± 6 | 24 ± 9 |
| (3-26 μmol/L) | BO | 7 ± 2 | 18 ± 3 | 25 ± 7 | 23 ± 5 |
| Direct bilirubin | MO | 2 ± 1 | 12 ± 7 | 11 ± 3 | 11 ± 7 |
| (0-5 μmol/L) | BO | 1 ± 0 | 3 ± 0 | 6 ± 2 | 9 ± 5 |

Legend: CPB, Cardiopulmonary bypass.

## Discussion

The goal of improving hemocompatibility of the extracorporeal circuit for cardiac operations is to prevent coagulation disorders which might be judged quantitatively by the postoperative blood loss and the associated need of blood transfusions. Introduction of improvements in certain components of the circuit should therefore result in less blood loss and fewer blood transfusions. On the basis of these criteria, no clinical studies so far could prove convincingly the superiority of MOs over BOs. This is true even though under strictly standardized animal experiments, with the only variable under study being the oxygenator, great improvements in platelet preservation and associated protection of the coagulation system could be demonstrated with an MO.[14] Recently it was shown that vigorous blood/air contact, introduced by suction, eliminated all the improvements obtained by the MO.[15] In the complex clinical situation, even more factors are likely to affect platelets or to cause otherwise increased blood loss and need for more blood transfusions. Patients undergoing cardiac operations were studied by means of a computerized program permitting data analysis of the major factors involved.[9] These appeared to be the type of operation, the perfusion time, and the amount of cardiotomy suction. A comparative clinical study between BOs and MOs should therefore be limited to these three aspects in the same category of patients.

In this study of patients operated upon for coronary artery diseases, blood loss and whole blood requirements were significantly less in patients perfused with MOs as compared to BOs. The implication is that hemostasis is better maintained after bypass with an MO.

During bypass, red blood cell damage, as reflected by the increased plasma hemoglobin values, was somewhat higher in the BO group, but not significantly improved with MO perfusion. This confirms the observations of others that red blood cell damage during cardiopulmonary bypass is not reduced by MO perfusion[1 5 16].

No differences occurred in the white blood cell counts between the groups, and the initial decrease is readily compensated. This phenomenon can be explained by the large reserve capacity of the marginating pool[17] as well as by the rapid process of leukopoesis. Morphologic or functional studies of white blood cells are probably more suitable to determine a certain effect of MO perfusion.[18 19]

The initial same decrease of platelet counts in both groups is to be attributed partly to hemodilution and partly to the severe aggregating effect of the first contact of blood with the extracorporeal circuit.[20] Platelet counts were consistently higher in the MO than in the BO group, although not significantly so.

More pertinent are the differences in platelet function between MO and BO perfusion. Although a substantial fall in function was experienced during the initial blood/circuit contact in both groups, the initial fall in function was less severe and transient in the MO group. The severe decrease in function in the MO group after 90 minutes of perfusion was undoubtedly the effect of increased suction (Fig.3), because it coincided with the release of the aortic cross-clamp. This observation substantiates our experimental results showing the deleterious effects of suction on the improvements that are obtained with MO perfusion[15]. In addition, the observation of a consistent decline of platelet function in both groups 30 minutes after perfusion is of importance.

It could be proved in a separate group of patients that this drop in function was associated with protamine administration. This theory fits in the frequent clinical observation of no diffuse bleeding with no visual clots directly after bypass, and then oozing in conjunction with clots after protamine had been given. This effect is mostly transient, as platelet function is restored after 1 hour. Of interest is that the less affected platelets during MO perfusion do regain better function than the more affected platelets during BO perfusion. This negative effect of protamine on hemostasis also has been reported by others.[20 21] Recent experimental studies have shown that this effect could be reduced or eliminated by giving the appropriate protamine dose to neutralize heparin.[22]

Theoretically, a combination of MO perfusion with controlled suction and proper dosage of protamine should result in optimal function of the platelets and better hemostasis.

Although the whole blood requirements of an average of about 5 units are comparable to international figures,[8 16 23] this does not necessarily mean that these are acceptable figures. With an optimal hemostatic capacity, blood loss probably can be minimized and blood requirements substantially reduced.

Cardiac operations can be performed without donor blood,[24] but not routinely as yet. Such procedures might become common in the future if, along with use of an MO, other blood damaging factors such as uncontrolled suction and overdosage of protamine can be eliminated in routine cardiac procedures.

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CHAPTER 4

# THE EFFECT OF PROSTAGLANDIN $E_1$ IN PATIENTS UNDERGOING CLINICAL CARDIOPULMONARY BYPASS

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## SUMMARY

The effect of prostaglandin $E_1$ ($PGE_1$) on protection of platelets during cardiopulmonary bypass (CPB) was evaluated in 9 patients, who were compared with an identical control group of 10 patients undergoing coronary artery bypass grafting. To evaluate the hemodynamic side-effects, $PGE_1$ (0.05 μg/kg/min) was infused prior to CPB, resulting in a 26% drop in mean systemic arterial pressure. With this dose, no inhibition of the adenosine diphosphate-induced aggregation could be measured in the pulmonary artery sample. During CPB, the same infusion dose resulted in a severe drop in systemic arterial pressure below 50 mm Hg in 7 of the 9 patients. In 5 of these patients, volume load and phenylephrine infusion could not compensate for the pressure drop, and $PGE_1$ had to be reduced to 0.02 μg/kg/min. Platelet aggregation was reduced significantly in the $PGE_1$-treated group compared with the control group, but not completely inhibited during CPB. However, in the postbypass period no platelet preservation was seen in the $PGE_1$ group. In both groups, platelet number and function were equally low. No differences were measured in blood loss or blood transfusion requirements. Except for hypotension, no side-effects of the $PGE_1$ treatment were seen. It is concluded that the hypotension caused by minimal doses of $PGE_1$ during CPB precluded using higher doses, which might have had a greater effect on platelet inhibition. These hypotensive side-effects should be reduced or eliminated before $PGE_1$ can be expected to have the effect on platelet damage that has been demonstrated in animal experiments.

## INTRODUCTION

The tendency toward postoperative bleeding in open-heart operations is associated with decreased numbers and impaired function of platelets[1,2]. This is the result of a continuous platelet adhesion and aggregation process, which is induced by the synthetic surface of the extracorporeal circuit and is not prevented by heparin anticoagulation. Several factors contribute to the damage to the platelets: the type of oxygenator[3,4], the oxygenator prime[5], the cardiotomy suction[6-11], and the administration of protamine[12]. The use of drugs that inhibit platelet aggregation has been shown to prevent, at least partly, the exhaustion of platelet function[13-20]. In cardiopulmonary bypass (CPB), however, drugs with short-term effects are required to obtain immediate complete reversal of platelet inhibition at the end of the procedure.

In animal experiments infusion of prostaglandin $E_1$ ($PGE_1$) during CPB has been shown to inhibit platelet function effectively, as well as being able to preserve platelets and restore normal hemostasis afterwards without impairing hemodynamics[15-17]. Our clinical study evaluates the hematological and hemodynamical effects of $PGE_1$ in patients undergoing CPB.

## PATIENTS AND METHODS

This prospective study included 19 patients requiring aortocoronary bypass grafting[21]. The results for 9 randomly selected patients who received $PGE_1$ treatment were compared with those for a control group of 10 patients (Table 1). In patients receiving coumarin derivatives, this treatment was adjusted to obtain Thrombotest (Nyegard, Oslo, Norway) values between 15 and 25% preoperatively.

Table 1. Clinical characteristics of study patients.

| Characteristic | Control group (n = 10) | $PGE_1$ group (n = 9) |
|---|---|---|
| Age (yr) | 53 ± 5 | 55 ± 6 |
| Weight (kg) | 79 ± 8 | 78 ± 9 |
| Bypass time (min) | 128 ± 22 | 124 ± 37 |
| Range | 81 — 155 | 77 — 176 |

* Values shown are mean ± standard deviation (SD).

Anesthesia was induced with etomidate (0.3 mg/kg, Janssen, Beerse, Belgium) and maintained with a nitrous oxide-oxygen mixture (inspired oxygen fraction, 0.5), morphine (2 mg/kg), pancuronium bromide (0.1 mg/kg), and droperidol (0.25 mg/kg). All patients were ventilated at normal breathing rates. Heparinization (300 IU/kg; Leo, Emmen, The Netherlands) was initiated prior to cannulation and readministered (100 IU/kg) hourly. The effect of heparin was reversed within 30 minutes following CPB using protamine hydrochloride (600 IU/kg; Hoffmann-La Roche, Mijdrecht, The Netherlands).

For cardiopulmonary support, the Travenol Total Bypass Membrane Oxygenator* with polypropylene membrane was used. Synchronized roller pumps** were utilized for the return of blood to the oxygenator and the aortic cannula. One pump was used for suction of left ventricular decompression and two for cardiotomy suction. For the standardized circuit[22], polyvinylchloride tubing was used; the tubing in the roller pump was silicon rubber.

The CPB circuits were primed with 2000 ml of Ringer's lactate and 500 ml of albumin (20%). Further amounts of this mixture, replenished with packed cells to maintain the hematocrit above 20%, were transfused when necessary during CPB. Blood obtained from suction was returned to the oxygenator through a cardiotomy reservoir incorporating a 27 $\mu$ filter (Bentley, Model Q-220F***). A flow transducer connected to a flowmeter integrator unit (Nycotron, Model 376/393)**** was placed in the outlet line of the cardiotomy reservoir to calculate the total amount of sucked blood. No filters were used in the arterial line.

* TMO, Travenol Laboratories, Inc., Deerfield, IL.
** Dreissen B.V., Hellevoetsluis, The Netherlands.
*** Bentley Laboratories, Inc., Santa Ana, CA.
**** Nycotron, Drammen, Norway.

Moderate hypothermia (28° C) was employed routinely. All temperatures were measured in the nasopharynx. The estimated pump flow rates were 3 L/min/m² of body surface area at normothermia, reduced to 2.4 L/min/m² at 28° C. Flow was further reduced when the oxygen saturation in the venous return line (In Vivo Haemoreflectometer)* was higher than 75%. Cardioplegia was achieved with the solution used by Bretschneider and colleagues[23] (20 ml/kg) at 4° C. This solution was sucked into a separate reservoir (Polystan, HL-287/DFA)**, from which the red blood cells could be returned to the patient after centrifugation. At the termination of bypass, the patient was rewarmed to 37° C.

One milliliter ampules containing $PGE_1$ (Upjohn, Kalamazoo, MI) in dehydrated alcohol (0.5 mg/ml) were diluted before use in 9 ml bacteriostatic water with benzyl alcohol to make 10 ml of solution containing 50 μg of $PGE_1$ per milliliter. For infusion, this solution was diluted in 5% glucose saline; the final concentration of $PGE_1$ was 1 μg/ml.

To measure the effect of $PGE_1$ on platelets and systemic blood pressure without the influence of extracorporeal circulation, an intravenous infusion of 0.05 μg of $PGE_1$ per kilogram per minute was given to the $PGE_1$-treated patients. This was continued for 10 minutes after cannulation, before the patient went on CPB. Because $PGE_1$ is metabolized mainly in the lung, samples for platelet function study were taken simultaneously from the pulmonary artery and the radial artery. Immediately following this 10-minute infusion period, the $PGE_1$ infusion site was changed to the line for return of venous blood to the oxygenator, and CPB was initiated. If during the $PGE_1$ infusion the arterial blood pressure dropped below 50 mm Hg, the pump flow was increased and volume replacement given if needed. If the blood pressure did not improve, an infusion of 2 μg/kg/min of phenylephrine was started. The $PGE_1$ infusion rate was diminished from 0.05 to 0.02 μg/kg/min only if this measure failed. The $PGE_1$ infusion was continued up to the end of CPB.

Blood loss, aspirated from the operation site and from chest tube drainage, was measured from the moment of protamine administration until 8:00 A.M. on the first postoperative day in the intensive care unit. Other blood losses (e.g., in gauzes) were not determined.

Transfusions of whole bank blood, drawn within 24 hours, and red blood cell concentrates were given when hemoglobin values dropped below 100

* Schwarzer, München, West Germany.

** Polystan, Herlev, Denmark.

gm per liter. Platelet concentrate, cryoprecipitate, and fresh frozen plasma were transfused when bleeding persisted.

Blood samples drawn from the radial artery were studied at frequent intervals up to the tenth postoperative day for red cell, white cell, and platelet counts (Trombocounter),* hematocrit, hemoglobin, plasma hemoglobin[24] fibrinogen[25], fibrinogen degradation products (Thrombo-Wellcotest)** and antithrombin III (Coatest).*** In vitro platelet aggregation was determined quantitatively by measuring the maximum changes in optical density of platelet-rich plasma by adding adenosine diphosphate (ADP) in a final concentration of 0.83 $\mu$g/ml and comparing it with platelet-poor plasma. The frequency of sampling made determination of the aggregation curve at different doses of ADP prohibitive. The relatively high final concentration of ADP was chosen so that aggregation could be measured at the end of the procedure after platelet function had been impaired. Platelet-rich plasma was prepared by centrifuging citrated blood at 1,200 rpm for 5 minutes. The remaining blood was centrifuged at 3,000 rpm for 10 minutes to obtain platelet-poor plasma. The optical density was measured in a Payton aggregation module[26].****

Blood urea nitrogen, serum creatinine, urine output, alkaline phosphatase, lactic dehydrogenase, glutamic and pyruvic transaminases, and total and direct bilirubin levels were measured routinely. The myocardial isoenzyme of creatine kinase (CK-MB) was also measured to assess damage to heart muscle tissue.

The course of each patient's central temperature was evaluated frequently up to 8:00 A.M. on postoperative day 1 (POD 1).

Data were processed using a Digital Equipment Corp. PDP 11/10 minicomputer. For statistical analysis of differences between the groups, the Student $t$ test was used, with $p$ values greater than 0.05 considered not significant (NS). Values are expressed either as a mean $\pm$ the standard error of the mean (SEM) or as a mean $\pm$ standard deviation (SD).

* Coulter Electronics Ltd, Dunstable, England.
** Welcome Reagents Ltd, Beckenham, England.
*** Kabi Diagnostica, Stockholm, Schweden.
**** Payton Associates Ltd, Scarborough, Ont., Canada.

## RESULTS

After infusion of 0.05 $\mu$g of $PGE_1$ per kilogram per minute for 10 minutes before CPB was started, the mean arterial blood pressure dropped significantly from 99 $\pm$ 6 mm Hg (SEM) to 73 $\pm$ 6 mm Hg ($p < 0.05$). In this 10-minute period, platelet aggregation was not reduced. Before the start of $PGE_1$ infusion, aggregation was 52 $\pm$ 19% (SD) and remained 49 $\pm$ 22% in the pulmonary artery and 54 $\pm$ 13% in the radial artery at 10 minutes of $PGE_1$ infusion.

After the start of CPB the mean arterial blood pressure dropped in 7 $PGE_1$-treated patients within 10 minutes to levels below 50 mm Hg. In these patients the pump speed was increased to maximum rates, extra volume was given, and 1 to 2 $\mu$g/kg/min of phenylephrine infused. In 5 of these patients, the $PGE_1$ infusion rate had to be reduced to 0.02 $\mu$g/kg/min to maintain blood pressures above 50 mm Hg.

The average pump flow was 2.5 $\pm$ 0.3 L/min/m$^2$ in the control group and 3.1 $\pm$ 0.3 L/min/m$^2$ in the $PGE_1$-treated group. The average total amount of cardiotomy suction and apex suction was 38 liters (range, 12 to 65 liters) in the control group, and 26 liters (range, 2 to 50 liters) in the $PGE_1$-treated group ($p$ = NS). After the release of the aortic cross-clamp, two to three times more suction was used than in the preceding bypass period.

The transfusion requirements of blood, blood products, and electrolyte solutions are give in Table 2. The requirements for whole donor blood were about the same for both groups. Red blood cell concentrate was given in 2 of 10 patients (1 and 2 U, respectively) in the control group, compared with 7 of 9 patients in the $PGE_1$-treated group. Fresh frozen plasma and platelet concentrate were given to 2 patients in the control group and to 1 patient in the $PGE_1$-treated group. Cryoprecipitate was given to only 2 patients in the control group. The total amount of electrolyte and albumin solutions given was about five to six times greater in the $PGE_1$ group to compensate for hypotension.

The average blood loss in the control and $PGE_1$ groups was 1.0 $\pm$ 0.3 liters and 1.1 $\pm$ 0.3 liters, respectively; this was not significant. Table 3 shows that there were no significant differences between the two groups for changes in hematocrit, hemoglobin or plasma hemoglobin level, red blood cell count, or white blood cell count.

Table 2. Transfusion requirements in the control and $PGE_1$ groups*.

| Requirement | Control group (n = 10) | $PGE_1$ group (n = 9) | Significance |
|---|---|---|---|
| Whole blood (U) | 4.7 ± 1.7 | 3.6 ± 1.8 | NS |
| Red blood cell concentrate (U) | 0.3 ± 0.7 | 1.6 ± 1.1 | NS |
| Platelet concentrate (U) | 0.8 ± 2.0 | 0.7 ± 2.0 | NS |
| Fresh frozen plasma (U) | 1.3 ± 2.3 | 0.4 ± 0.8 | NS |
| Cryoprecipitate (U) | 2.9 ± 6.7 | 0 | |
| Human albumin (ml) | 430 ± 337 | 2421±1171 | $p < 0.01$ |
| Electrolyte solutions (ml) | 824 ± 839 | 4755±1693 | $p < 0.01$ |

* Values shown are mean ± SD.
U = donor units; NS = not significant.

Table 3. Changes in hematological variables in the control (n = 10) and $PGE_1$-treated (n = 9) groups*.

| Variable | Pre-CPB | 5 min CPB | End CPB | 2 hr Post-CPB | POD 7 |
|---|---|---|---|---|---|
| Hematocrit (42-52%) | | | | | |
| Control group | 36 ± 1 | 23 ± 1 | 22 ± 1 | 30 ± 3 | 37 ± 1 |
| $PGE_1$ group | 39 ± 2 | 25 ± 1 | 22 ± 1 | 30 ± 1 | 37 ± 1 |
| Hemoglobin (140-170 gm/L) | | | | | |
| Control group | 123 ± 4 | 80 ± 5 | 73 ± 3 | 97 ± 8 | 126 ± 6 |
| $PGE_1$ group | 132 ± 5 | 88 ± 3 | 77 ± 2 | 96 ± 5 | 123 ± 5 |
| Plasma hemoglobin (< 5 mg%) | | | | | |
| Control group | 14 ± 1 | 18 ± 4 | 37 ± 5 | 34 ± 6 | < 5 |
| $PGE_1$ group | 13 ± 3 | 27 ± 5 | 41 ± 6 | 41 ± 11 | < 5 |
| Red blood cell count ($4.6\text{-}6.2 \times 10^{12}$/L) | | | | | |
| Control group | 3.8 ± 0.1 | 2.6 ± 0.2 | 2.4 ± 0.1 | 3.2 ± 0.2 | 3.9 ± 0.1 |
| $PGE_1$ group | 4.0 ± 0.1 | 2.7 ± 0.1 | 2.4 ± 0.1 | 3.2 ± 0.1 | 3.7 ± 0.1 |
| White blood cell count ($4\text{-}11 \times 10^9$/L) | | | | | |
| Control group | 5.9 ± 0.5 | 4.0 ± 0.5 | 6.1 ± 1.0 | 6.6 ± 0.9 | 10.0 ± 1.1 |
| $PGE_1$ group | 6.6 ± 0.5 | 4.3 ± 0.6 | 8.7 ± 1.3 | 9.8 ± 1.7 | 10.4 ± 1.0 |

* Values shown are mean ± SEM; values in parentheses are normal values.
$PGE_1$ = prostaglandin $E_1$; CPB = cardiopulmonary bypass; POD = postoperative day.

Mean prebypass platelet aggregation (normal range, more than 60%; Fig. 1) decreased in both groups from about 50 to 60% to about 38% within 5 minutes after the start of bypass. Platelet aggregation remained at this level in the control group, while the aggregation in the $PGE_1$-treated group further decreased to 19 ± 4% and stabilized. Differences between both groups were significant at 30, 60, 90, and 120 minutes after the start of bypass. After the end of bypass and after administration of protamine, platelet aggregation decreased to less than 10% in both groups and remained at this level for the following hour.

Platelet aggregation began to recover in the control group 120 minutes following termination of CPB, while in the $PGE_1$-treated group recovery began between 120 minutes and one day following termination of CPB. On POD 1 platelet aggregation was statistically significant higher in the control group. Thereafter the changes in both groups followed the same course.

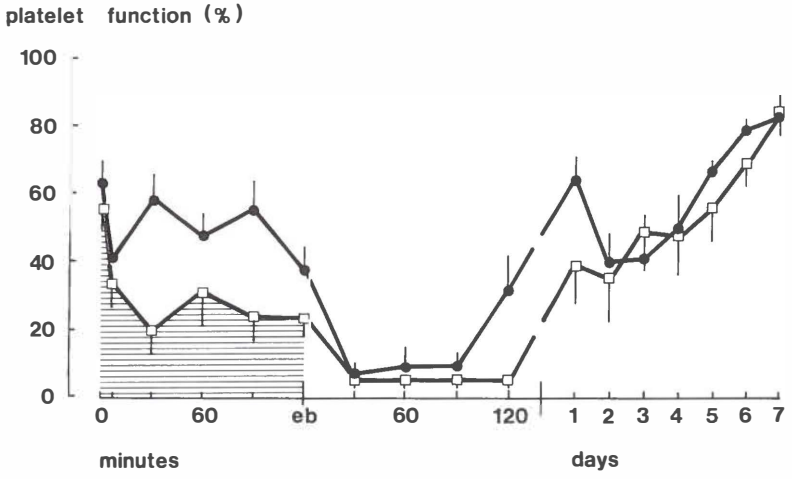


Fig. 1. The effect of cardiopulmonary bypass (CPB) on platelet function (maximum optical density of adenosine diphosphate-induced aggregation) in 9 patients treated with prostaglandin $E_1$ ($PGE_1$) (□——□) and 10 control patients (●——●). The points represent means; the error bar denotes one standard error of the mean (SEM). eb = end of bypass.

Mean preoperative platelet count was 210 × $10^9$/L (normal range, 150 × $10^9$ to 350 × $10^9$/L; Fig. 2). There was a decrease in both groups to 169 × $10^9$/L during prebypass surgery. The platelet count declined to about 110 × $10^9$/L at 5 minutes and reached a minimum level of 90 × $10^9$/L (43%) after 30 minutes of CPB. Recovery was not seen in either of the groups before POD 4. From this day on, platelet counts increased almost linearly to values of about 375 × $10^9$/L (185%) on POD 7. No significant differences were noted between the groups.

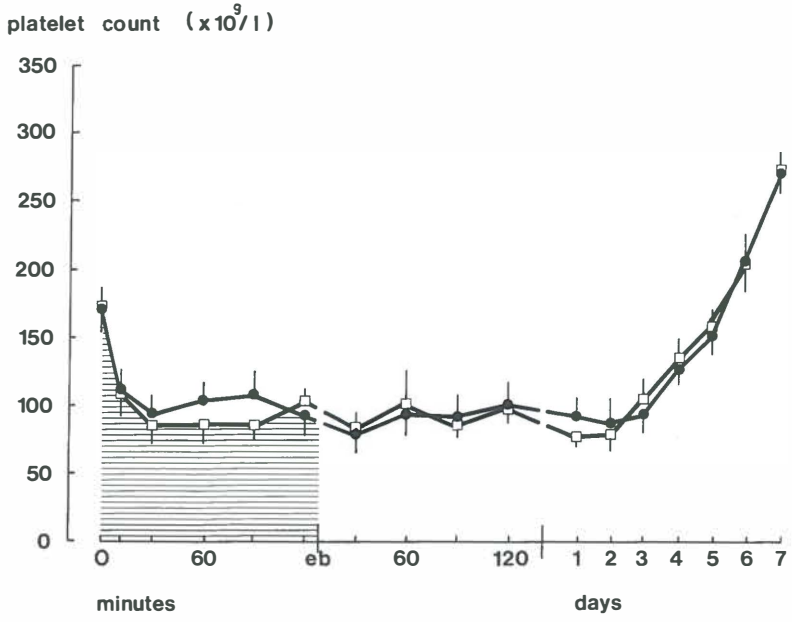


Fig. 2. The effect of membrane oxygenator perfusion on platelet counts in $PGE_1$-treated and control patients. (See Fig. 1 for explanation of other symbols).

Fibrinogen levels (normal range, 2 to 4 gm/L, Fig. 3) averaged 4.6 ± 0.5 gm/L in the control group and 4.0 ± 0.2 gm/L in the $PGE_1$-treated group prior to institution of CPB. A decrease in both groups to 2.2 ± 0.2 gm/L was measured at 90 minutes following bypass. These levels increased to a maximum on POD 4 of 7.5 ± 0.5 gm/L in the control group and 6.0 ± 0.7 gm/L in the $PGE_1$-treated group in the subsequent postoperative days. Throughout the postoperative period, fibrinogen levels were significantly higher in the control group compared with those in the $PGE_1$-treated group. Fibrinogen degradation products remained within the normal range (0 to 10 mg/L) in the control group. This variable increased to 27 ± 7 mg/L in the $PGE_1$-treated group at 90 minutes postbypass. Thereafter, fibrinogen degradation products increased only slightly (10 to 20 mg/L) in both groups up to POD 5. A further increase to 22 and 27 mg/L was seen in the control and $PGE_1$-treated groups, respectively, on POD 7.

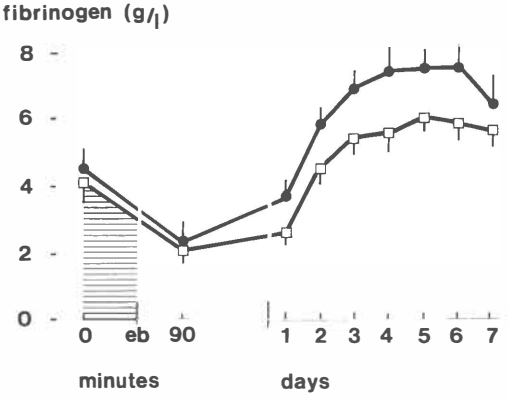


Fig. 3. The effect of membrane oxygenator perfusion on fibrinogen levels in $PGE_1$-treated and control patients. (See Fig. 1. for explanation of other symbols).

Antithrombin III (normal range, 95 to 120%) averaged 78 ± 10% and 63 ± 7% in both the control group and the $PGE_1$-treated group at 90 minutes postbypass. Antithrombin III remained at this level on POD 1 but increased thereafter in both groups, reaching the normal range on the POD 5.
Table 4 shows that there were no significant differences between the two groups for the course of blood urea nitrogen, serum creatinine, alkaline phosphatase, lactate dehydrogenase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, and total and direct bilirubin levels.

Table 4. Changes in variables reflecting kidney and liver function.

| Variable | Pre-CPB | 2 hr Post-CPB | POD 1 | POD 3 | POD 7 |
|---|---|---|---|---|---|
| Blood urea nitrogen (3.3-6.7 mmol/L) | | | | | |
| Control group | 5.6 ± 1.0 | 5.3 ± 0.1 | 5.2 ± 0.3 | 8.8 ± 0.5 | 10.5 ± 1.6 |
| $PGE_1$ group | 5.8 ± 0.4 | 5.1 ± 1.2 | 4.4 ± 0.4 | 8.1 ± 1.1 | 7.3 ± 0.5 |
| Serum creatinine (62-106 μmol/L) | | | | | |
| Control group | 83 ± 13 | 83 ± 15 | 92 ± 5 | 118 ± 17 | 123 ± 43 |
| $PGE_1$ group | 93 ± 4 | 132 ± 13 | 105 ± 11 | 100 ± 7 | 86 ± 3 |
| alkaline phosphatase (13-120 U/L) | | | | | |
| Control group | 71 ± 4 | 40 ± 6 | 51 ± 7 | 86 ± 10 | 152 ± 46 |
| $PGE_1$ group | 80 ± 7 | 50 ± 3 | 28 ± 5 | 60 ± 5 | 89 ± 16 |
| Lactate dehydrogenase (114-235 U/L) | | | | | |
| Control group | 172 ± 19 | 336 ± 39 | 346 ± 19 | 330 ± 21 | 333 ± 13 |
| $PGE_1$ group | 208 ± 12 | 332 ± 8 | 331 ± 10 | 388 ± 21 | 346 ± 23 |
| Glutamic oxalacetic transaminase (< 40 U/L) | | | | | |
| Control group | 23 ± 2 | 53 ± 5 | 47 ± 7 | 46 ± 6 | 40 ± 4 |
| $PGE_1$ group | 23 ± 2 | 72 ± 21 | 55 ± 3 | 54 ± 6 | 33 ± 5 |
| Glutamic pyruvic transaminase (< 30 U/L) | | | | | |
| Control group | 21 ± 5 | 16 ± 3 | 18 ± 2 | 35 ± 8 | 53 ± 9 |
| $PGE_1$ group | 23 ± 3 | 18 ± 2 | 19 ± 1 | 27 ± 2 | 52 ± 10 |
| Total bilirubin (3-26 μmol/L) | | | | | |
| Control group | 7 ± 1 | 21 ± 5 | 36 ± 6 | 31 ± 11 | 24 ± 9 |
| $PGE_1$ group | 9 ± 2 | 21 ± 7 | 22 ± 2 | 14 ± 2 | 10 ± 1 |
| Direct bilirubin (0-5 μmol/L) | | | | | |
| Control group | 2 ± 1 | 4 ± 1 | 11 ± 3 | 17 ± 11 | 11 ± 7 |
| $PGE_1$ group | 3 ± 1 | 10 ± 8 | 5 ± 1 | 4 ± 1 | 2 ± 1 |

* Values shown are mean ± SEM; values in parentheses are normal ranges.
CPB = cardiopulmonary bypass; Pre-CPB = within 30 minutes before CPB; POD = post-operative day.

Average urine production during CPB was 5.7 ml per minute in the control group and 5.1 ml per minute in the $PGE_1$-treated group; for the first 30 minutes after bypass, urine production was 8.3 ml per minute and 10.1 ml per minute, respectively. Until 8:00 A.M. on POD 1 in the intensive care unit, an average of 1,745 ± 530 ml was produced by the control group and 1,993 ± 639 ml by the $PGE_1$-treated group. None of the differences between the groups was statistically significant.

From gastric tubes in the intensive care unit, an average of 212 ± 170 ml was drained in the control group and 739 ± 263 ml in the $PGE_1$-treated group ($p$ = NS).

The myocardial isoenzyme of creatine kinase (CK-MB) was increased (more than 20 IU per liter) during the postoperative course in 1 patient in each group.

The postoperative central temperature increased initially above 38.9°C in 6 patients in the control group and in 7 $PGE_1$-treated patients, but returned before 8:00 A.M. on POD 1 to values below 39°C in all patients.

## DISCUSSION

The potential beneficial effect of $PGE_1$ to reduce damage to platelets during extracorporeal circulation has been reported since 1973[19]. This prostaglandin preserves platelet counts during in vitro recirculation of human blood employing membrane oxygenators, reduces the development of platelet factor 4, maintains structural morphology, and preserves sensitivity to aggregating agents[15]. Furthermore, during extracorporeal oxygenation in dogs[16] as well as in monkeys[17], $PGE_1$ preserves platelet numbers and function, which are associated with restoration of bleeding times to normal levels postoperatively.

In this study the effect of $PGE_1$ in patients on CPB was evaluated. Infusion of $PGE_1$ before CPB and during intact pulmonary circulation resulted in an impressive decrease of the systemic blood pressure. This was induced by a relatively small dose of $PGE_1$, which had a minimal inhibiting effect on platelet function. With a similar small dose during CPB, however, platelet function was more inhibited. This is most likely explained by the fact that $PGE_1$ is metabolized primarily in the lung, which is bypassed during CPB[27]. The dose of $PGE_1$ that could be infused during CPB was limited considerably by its severe hypotensive side-effect. The hypotension could be managed only by increased pump flow rates, additional circulating volume, and the use of phenylephrine as a vasoconstrictor. Despite all these measures the

initial dose of 0.05 μg of $PGE_1$ per kilogram per minute had to be decreased to 0.02 μg/kg/min in about half of the patients. In animal experiments with $PGE_1$ infusion during extracorporeal circulation[16,17], much larger doses could be given: more than 20 to 40 times higher in dogs and 4 to 100 times higher in monkeys than in human beings. These high doses had only moderate hypotensive effects in the animals and phenylephrine treatment was never required. Although the experimental studies were performed under normothermia instead of hypothermia in the clinical situation, this does not explain the different results. Differences in sensitivity for the vasodilating effect of $PGE_1$ in various species might be of prime importance. With the substantially reduced dose of $PGE_1$ in human beings, the favorable effects of platelet inhibition could not be reproduced. The $PGE_1$-treated patients did not show better preservation of platelets, and in the postbypass period platelet function remained even lower compared with that in the control group.

In contrast, platelet numbers in the animal experiments were better preserved. Moreover, platelet function had recovered in the $PGE_1$-treated group by the postbypass period, and this recovery was associated with restoration of normal bleeding times. In our clinical study no improvement in platelet behavior was obtained. There were no significant differences between the groups in postoperative blood requirements or blood loss. The only significant differences noted were in the electrolyte and albumin solutions given; this was related to the hypotensive effect of $PGE_1$. The prostaglandin had little or no effect on the other hematological variables. It did not influence fibrinogen depletion, although a lower reactive increase of fibrinogen levels was noted during the first postoperative week. While this less pronounced reactive increase of fibrinogen in the first week is obscure, it does suggest either reduced overall fibrinogen depletion or a suppressed stimulus for fibrinogen production. No effect of the $PGE_1$ treatment was seen on the red or white blood cells, the course of kidney and liver function, damage to heart tissue, or the postoperative temperature course.

From this clinical study, it can be concluded that $PGE_1$ in human beings has a strong vasodilatory effect that prevents the administration of a sufficiently high dose to inhibit platelet function effectively. This limiting hypotensive side-effect must be overcome before the protective effect obtained with a higher dose during clinical CPB can be expected. In our experimental studies[28], we have already found that prostacyclin offers also a platelet protecting capability, at an equal blood pressure reducing dosage. Clinical studies have yet to confirm the effectiveness of prostacyclin in this application.

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CHAPTER 5

# HEMODYNAMIC SIDE-EFFECTS OF PROSTAGLANDIN $E_1$ IN PATIENTS BEFORE AND DURING CARDIOPULMONARY BYPASS

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**SUMMARY**

Animal experiments have shown that the administration of prostaglandin $E_1$ ($PGE_1$) during cardiopulmonary bypass (CPB) reduces platelet damage and the tendency to bleeding disorders.

Because $PGE_1$ also has a strong vasodilating action, a clinical trial was started to evaluate its hemodynamic side-effects. $PGE_1$ was studied during 3 different periods:

1. a 10-minute period before bypass,
2. a hemodynamically stable period during CPB,
3. during the whole period of CPB.

1. Before CPB, a consistent fall of mean arterial pressure (MAP) was observed (26 mm Hg). 2. During the stable period of CPB, the blood pressure fall was dependent on the pre-existing systemic vascular resistance. 3. $PGE_1$ infusion during the whole CPB period was, in general, badly tolerated. Increased perfusion flow was not sufficient to compensate for the resulting blood pressure drop. In most of the 13 cases vasopressors had to be given continuously. In 7 patients $PGE_1$ had to be reduced, and in 4 patients the infusion had to be stopped altogether.

Because of the marked vasodilator effects of $PGE_1$ it appears impossible to give patients doses adequate to achieve platelet preservation during clinical CPB with safety.

**Key-Words:** Open heart surgery — Prostaglandin — $PGE_1$ — Hemodynamic.

## INTRODUCTION

Despite improvements in cardiopulmonary bypass (CPB) techniques, bleeding disorders still occur frequently. The reason for this is that the non-physiological surfaces stimulate the platelets to aggregate, a process which is not prevented by heparinization, consequently resulting in a substantial loss of platelet function at the end of CPB. In animal experiments platelet-aggregation inhibition by prostaglandin $E_1$ ($PGE_1$) was found to prevent platelet damage during bubble oxygenator perfusion and normal bleeding times were obtained after bypass[1,6]. These favorable results stimulated us to study the effects of $PGE_1$ as a platelet protective agent during CPB in patients. Its hypotensive side-effects were recognized but were easy to manage in the dog experiments. Although the cardiovascular actions of $PGE_1$ have been studied intensively in healthy volunteers[2-4], nothing is known about its effects on anaesthetized patients with cardiac disease.
In a recent clinical trial of the platelet-protecting properties of $PGE_1$ during and after CPB in our clinic more extensive hemodynamic studies have been performed and are presented here.

## METHODS

Anesthesia was induced with etomidate (Hypnomidate®)* and maintained with morphine (< 3 mg/kg), Dehydrobenzperidol®* (0.25 mg/kg), pancuroniumbromide (Pavulon®)** and nitrous oxide ($FIO_2 = 0.5$). For controlled ventilation ($FECO_2 = 0.05$) a Servo Ventilator 900B*** was used in conjunction with a $CO_2$-Analyzer (Siemens 930)***.
Nitroglycerine (0.2 mg) was given intravenously, if needed, during and after sternotomy. No other drugs were given except heparine**** (3 mg/kg) within 30 minutes prior to the hemodynamic studies with $PGE_1$.
The paired t-test was used for statistical analysis of the results. Significance was assumed at the 5% level of probability ($p < 0.05$). The hemodynamic

* Janssen Pharmaceutica, Beerse, Belgium
** Organon, Oss, The Netherlands
*** Siemens Elema, Solna, Sweden
**** Leo, Emmen, The Netherlands

parameters measured, methods, equipment and the calculations used are shown in Table 1.

Table 1. Parameters measured, equipment, calculations and abbreviations.

| Parameters | Abbreviation | Unit | Device/method |
|---|---|---|---|
| Heart Rate | HR | $min^{-1}$ | Hewlett Packard 78209 A heart rate monitor[1] |
| Systolic arterial pressure | Psyst | mm Hg | Statham P 23 ID pressure transducer[2] used with Hewlett Packard pressure monitor 78205 B[1] |
| Diastolic arterial pressure | Pdiast | | |
| Mean arterial pressure | P art | | |
| Left atrial pressure | $\bar{P}$LA | | |
| Right atrial pressure | $\bar{P}$RA | | |
| Systolic pulmonary artery pressure | PAPsyst | | KMA 9601-7F Swan-Ganz-catheter[3] Pressure measurements were performed at the end of the expiration Recorder: Hewlett Packard 7748B[1] |
| Diastolic p.a. pressure | PAPdiast | | |
| Mean p.a. pressure | PAP | | |
| Left ventricular pressure | PLV | | Millar 6F Cathetertip transducer[4] used with a modified Hewlett Packard pressure monitor 78205 B[1] |
| Left ventricular end-diastolic pressure | PLVED | | |
| Left ventricular dp/dt max. | LV dp/dt max | mm Hg $s^{-1}$ | Recorder: Bell and Howell UV-recorder Nr. 5-137-P9—12[5] |
| Cardiac output | CO | l $min^{-1}$ | KMA 3500 E cardiac output computer[3] (thermodilution) |
| Cardiac index (CO: body-surface area) | CI | l $min^{-1}$ $m^{-2}$ | Injectate: 10 cc saline at 22°C. The average from at least 2 injections was calculated |
| Nasopharyngeal temperature | Tnp | °C | Yellow Springs Instruments 400 series[6] used with Hewlett Packard monitor 78124 A/200 |
| Skin temperature at left thenar eminence | Tskin | | |

| | | | |
|---|---|---|---|
| $CO_2$-minute production (per square meter at BTPS) | $VCO_2$ | ml $min^{-1}$ $m^{-2}$ | Siemens Elema 930 $CO_2$-Analyzer (in conjunction with Servo 900 B Ventilator) |

Further calculations:

$$\text{Stroke volume index (SVI) } (ml\ m^{-2}) = \frac{CI}{HR} \times 1000$$

$$\text{Systemic vascular resistance (SVR) } (MN\ s\ m^{-5}) = \frac{Part - PRA}{CO} \times 8$$

$$\text{Pulmonary vascular resistance (PVR) } (MN\ s\ m^{-5}) = \frac{PAP - PLA}{CO} \times 8$$

$$\text{Rate pressure product} = Psyst \times HR \quad (mm\ Hg\ min)$$

$$T\ (°C) = Tnp - Tskin$$

[1] Hewlett Packard Benelux, Amstelveen, The Netherlands
[2] Gould Godard, Bilthoven, The Netherlands
[3] Kimray Medical Association, Oklahoma City, USA
[4] Millar Instruments, Houston, Texas, USA
[5] Bell & Howell, Rotterdam, The Netherlands
[6] Yellow Springs Instrument Co., Yellow Springs, Ohio, USA

## 1. Hemodynamic effects of $PGE_1$ before CPB

Stabilization of the circulation was waited for after routine cannulation of the ascending aorta and insertion of cannulae in both venae cavae. In 10 male patients (Table 2) undergoing coronary revascularization $PGE_1$ (50 ng $kg^{-1}$ $min^{-1}$)* was infused for a 10-minute period and cardiovascular changes, including left ventricular pressure, were recorded.

Table 2. Data of patients investigated (mean ± SD)

| | Male/female | Age (years) | Weight (kg) | Height (cm) |
|---|---|---|---|---|
| 1. $PGE_1$ before CPB | 10/0 | 55±8 | 76±10 | 177±6 |
| 2. $PGE_1$ on CPB | 16/8 | 51±10 | 71±9 | 173±6 |
| 3. Continuous $PGE_1$ | 12/1 | 56±8 | 75±10 | 175±7 |

* The Upjohn Company, Kalamazoo, Michigan, USA

## 2. Hemodynamic effects of $PGE_1$ during CPB

An infusion of $PGE_1$ (50 ng $kg^{-1}$ $min^{-1}$ for 10 minutes) was administered to another group of 24 adult patients while on cardiopulmonary bypass during a hemodynamically stable period after aortic cross-clamping and cardioplegic[9] arrest of the heart.

The patients in this group (Table 2) underwent either coronary artery surgery or valve replacement procedures. Either Travenol-TMO oxygenators* or Sci-Med Spiral Coil Membrane lungs** were used, primed with a 5% human albumin solution in Ringer-lactate. The hematocrit was maintained between 20 and 25. The pump flow ($2.8 \pm 0.5$ $l m^{-2}$ $min^{-1}$) and nasopharyngeal temperature ($26.6 \pm 1.6°C$) were kept constant. Arterial and superior vena cava pressures were continuously recorded before and during $PGE_1$ administration, as well as during the 10 minutes after discontinuation of the infusion.

## 3. Hemodynamic effects of continuous administration of $PGE_1$ during the whole CPB procedure

In order to try to prevent platelet damage, $PGE_1$ was infused during the whole bypass period in a total of 13 patients (Table 2) with coronary artery disease. Perfusions were performed in the same manner as described above, but only Travenol-TMO oxygenators were used.

The aim was to administer $PGE_1$ in a dose of 50 ng $kg^{-1}$ $min^{-1}$ or more. If accompanying vasodilation resulted in mean arterial blood pressures below 50 mm Hg, the pump flow was to be increased and, eventually a vasoconstrictor drug (phenylephrine) administered if necessary.

* Travenol Laboratories, Morton Grove, Ill. USA

** Sci-Med Life Systems, Minneapolis, USA

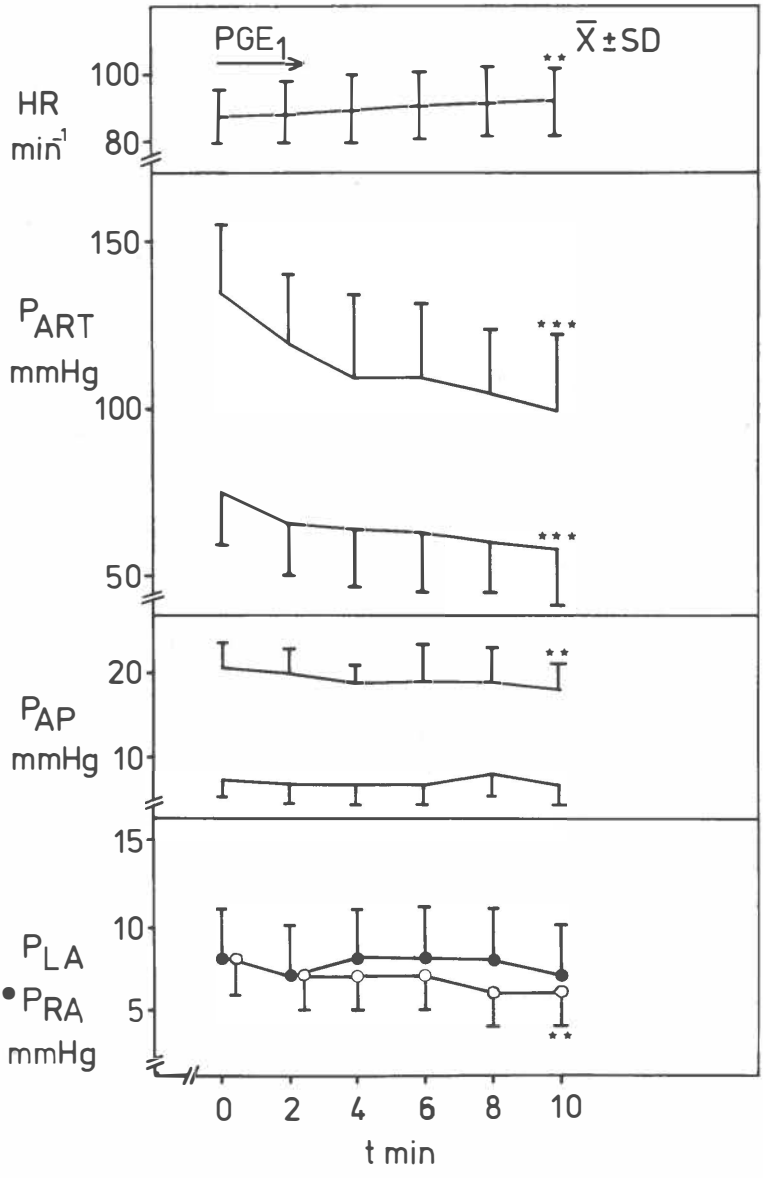


Fig. 1. Composite illustration of heart rate (HR), systolic and diastolic arterial pressures (PART), systolic and diastolic pulmonary artery pressures (PAP), right atrial pressure (PRA) and pulmonary capillary wedge pressure (PLA) during infusion of 50 ng $min^{-1}$ $kg^{-1}$ of $PGE_1$ before CPB. Degree of statistical significance against 0-values: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

## RESULTS

### 1. Hemodynamic effects of $PGE_1$ before CPB (Fig. 1, 2 and 3)

The measured hemodynamic parameters are presented in Fig. 1. Heart rate increased slightly (88 ± 8 to 92 ± 10 $min^{-1}$, $p < 0.01$). Systolic, mean and diastolic arterial pressures decreased markedly. With the dose of 50 ng $kg^{-1}$ $min^{-1}$, mean arterial pressure dropped from 99 ± 17 to 73 ± 18 mm Hg (—26 mm Hg, i.e. by 23%). A significant decrease was also seen in the systolic pulmonary artery pressure (21 ± 3 to 19 ± 3 mm Hg) and the pulmonary capillary wedge pressure (8 ± 2 to 6 ± 1 mm Hg). No significant changes were observed in the diastolic pulmonary artery pressure (7 ± 2 to 6 ± 2 mm Hg), or the right atrial pressure (8 ± 3 to 7 ± 3 mm Hg).

The derivatives of the hemodynamic parameters are presented in Fig. 2. The changes in heart rate and systolic arterial pressure resulted in a 25% fall in the rate-pressure product (12,100 ± 2,700 to 9,100 ± 2,700). Cardiac index, initially 3.0 ± 0.6 L $min^{-1}$ $m^{-2}$, was reduced by only 6% (to 2.8 ± 0.6 L $min^{-1}$ $m^{-2}$; $p < 0.05$).

The corresponding fall in stroke volume index was 9%. Systemic vascular resistance decreased by 23% ($p < 0.01$), but no gain in skin temperature was noted.

Pulmonary vascular resistance, initially 7.2 ± 8.9 MN s $m^{-5}$, showed a large variation and no trend was detectable. The left ventricular pressure fell (Fig. 3) corresponding with the drop in systolic arterial pressure; however, the enddiastolic pressure in the left ventricle did not change significantly. LV dp/dt decreased (1,500 ± 390 to 1,300 ± 360 mm Hg $s^{-1}$), but not the quotient dp/dt/IP (Veragut-Krayenbühl-Index[10]).

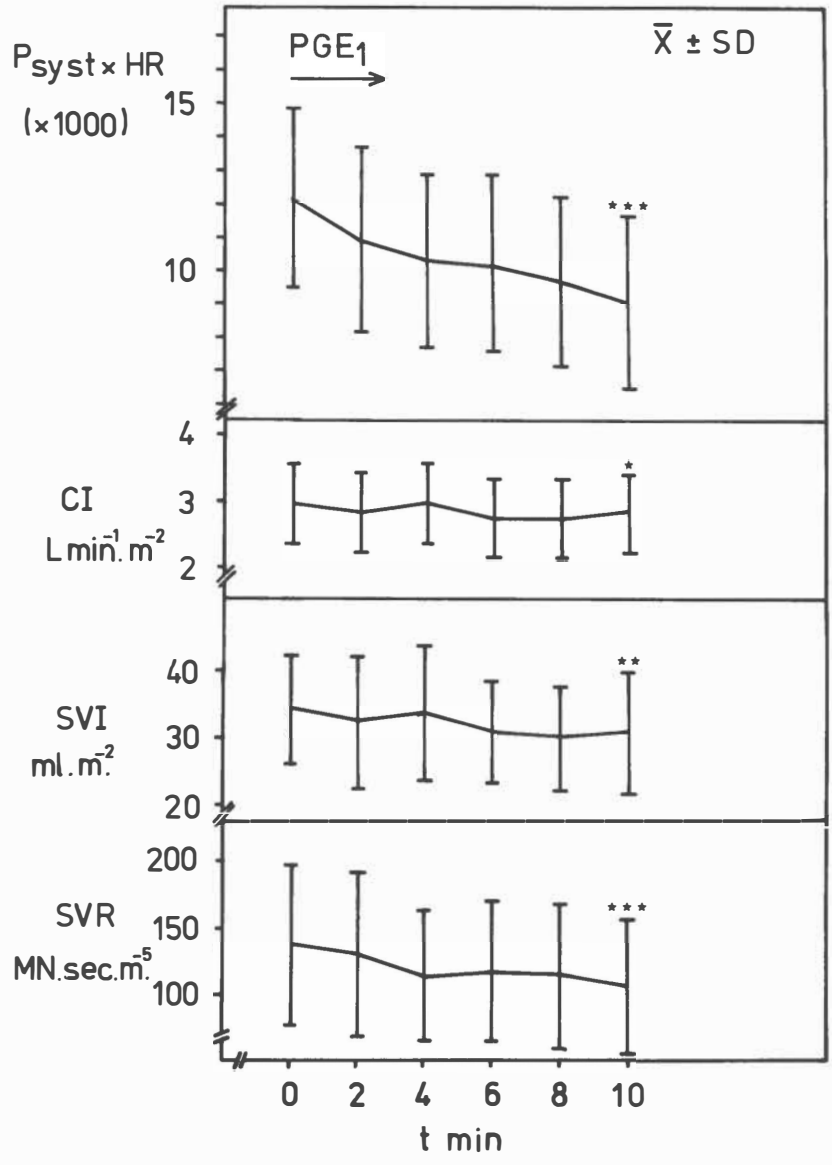


Fig. 2. Changes in rate pressure product ($P_{syst} \times HR$), cardiac index (CI), stroke volume index (SVI) and systemic vascular resistance (SVR) during infusion of $PGE_1$ (50 ng $kg^{-1}$ $min^{-1}$) before CPB.
Degree of statistical significance against O-values: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

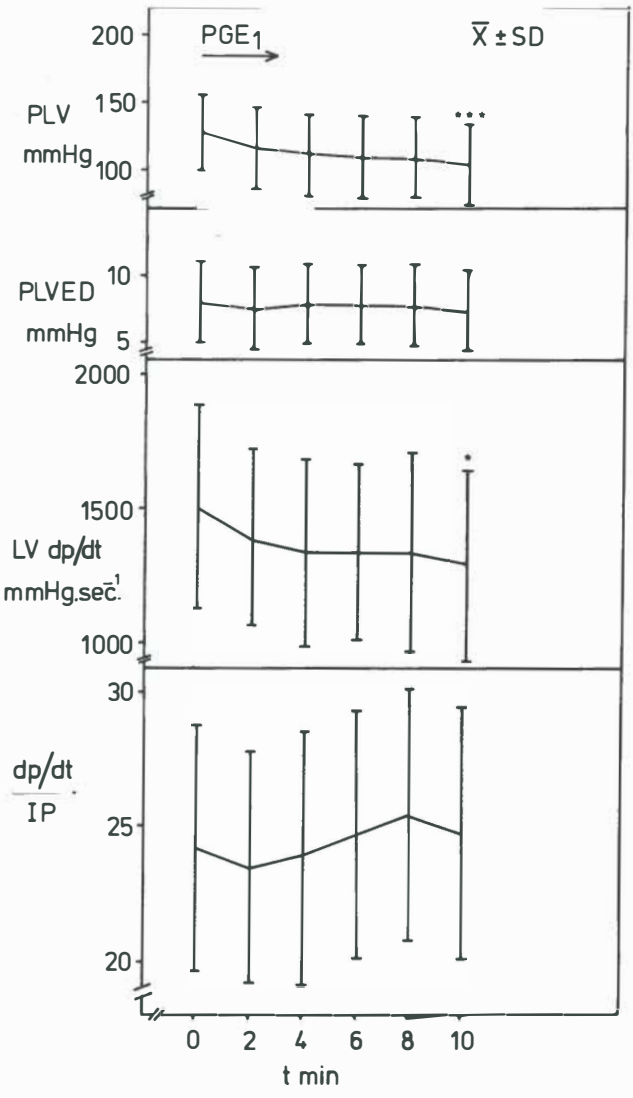


Fig. 3. Changes in left ventricular hemodynamics during infusion of $PGE_1$ ($50\,ng\,kg^{-1}\,min^{-1}$) before CPB.
PLV = systolic left ventricular pressure; PLVED = left ventricular enddiastolic pressure; LV dp/dt = maximal gain of left ventricular pressure; $\frac{dp/dt}{IP}$ = Veragut-Krayenbühl Index[10]
Degree of statistical siginificance against 0-values: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

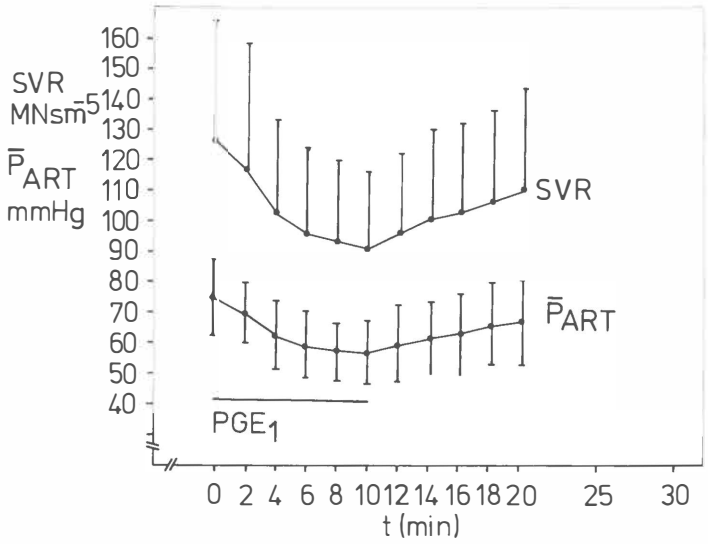


Fig. 4. Influence of $PGE_1$ (50 ng kg$^{-1}$ min$^{-1}$) during cardiopulmonary bypass on systemic vascular resistance (SVR) and mean perfusion pressure (PART).

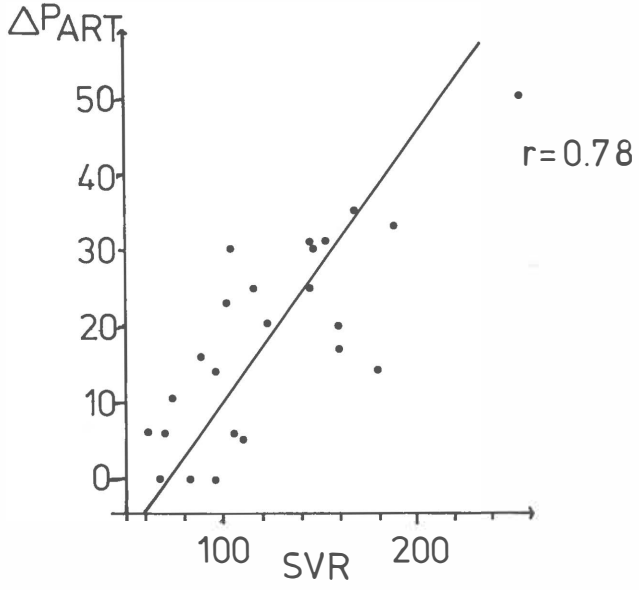


Fig. 5. Relation between pre-existing systemic vascular resistance (SVR) and drop in perfusion pressure caused by infusion of $PGE_1$ (50 ng kg$^{-1}$ min$^{-1}$) during CPB.

## 2. Hemodynamic effects of $PGE_1$ during CPB

During steady-state hypothermic CPB, $PGE_1$ leads to a significant reduction in arterial blood pressure ($p < 0.001$) and systemic vascular resistance ($p < 0.001$) within 10 minutes (Fig. 4). The mean pressure dropped from $74 \pm 11$ to $57 \pm 10$ mm Hg (—17 mm Hg, i.e. — 23%). When the infusion was discontinued, arterial pressure recovered promptly, but did not return to the initial values within 10 minutes. The extent of the blood pressure fall varied greatly. In 3 of the 24 patients the blood pressure did not fall at all and in 4 patients the maximum fall was only 10 mm Hg. In most instances, however, the blood pressure dropped between 10 and 30 mm Hg. Only in 3 patients did the mean blood pressure decrease by more than 30 mm Hg. In 7 out of 24 patients the dose of 50 ng $kg^{-1}$ $min^{-1}$ $PGE_1$ led to a transient fall of blood pressure to below 50 mm Hg. In none of the patients had the perfusion flows to be increased or vasopressors given. There was a relationship between the pre-existing systemic vascular resistance and the drop in blood pressure (Fig. 5): a high systemic vascular resistance was correlated ($r = 0.78$) with a more severe fall in arterial pressure.

### 3. Hemodynamic effects of the continuous administration of $PGE_1$ during the whole CPB procedure

In all the patients in this group the perfusion flow had to be considerably increased to compensate for a severe pressure drop at the beginning of the perfusion and in the rewarming period. Only in one out of 13 patients could the full dose of 50 ng $kg^{-1}$ $min^{-1}$ $PGE_1$ be continued without the application of a vasopressor; in the other 12 patients mean blood pressures fell to values below 50 mm Hg despite maximal perfusion flow (6.5 l/min). In 5 of these patients blood pressures could be restored above this level with the infusion of phenylephrine (0.04 - 1.4 mcg $kg^{-1}$ $min^{-1}$). In another 3 patients phenylephrine (0.7 - 1.4 mcg $kg^{-1}$ $min^{-1}$) failed to maintain adequate blood pressures and $PGE_1$ had to be reduced to 20 ng $kg^{-1}$ $min^{-1}$. In the remaining 4 patients even this treatment was not sufficient and, despite increasing the dose of phenylephrine (0.8 - 2.3 mcg $kg^{-1}$ $min^{-1}$) the $PGE_1$ infusion had to be stopped. One of these 4 patients developed a metabolic acidosis at the end of perfusion, which rarely occurs in our adult perfusions otherwise.
Severe hypotension was mainly experienced during nonstable hemodynamic periods: first, at the start of CPB in the period of transition from partial to total bypass (but in this period $PGE_1$ never had to be stopped); second, during rewarming and after release of the aorta cross clamps (when severe hypotension was the rule and $PGE_1$ had to be discontinued in 4 cases).

## DISCUSSION

Arterial hypotension was the common finding in our study, and this is in accordance with the literature. However, we did not observe an increase in cardiac output as described by Carlson[4]. We found a decrease in left ventricular dp/dt, in contrast to the observations of Nakano[8] in dogs. Our finding of decreasing dp/dt must not be interpreted as a negative inotropic effect, because aortic pressure fell concommitantly, and the Veragut-Krayenbühl-Index[10] $\frac{dp/dt}{IP}$ remained constant.
In contrast to the marked effects of $PGE_1$ on the systemic circulation, the effects on the pulmonary and venous circulation were less pronounced.
The hemodynamic changes of 50 ng $kg^{-1}$ $min^{-1}$ $PGE_1$ were not essentially different in the stable periods prior to and during bypass. A rapid decrease of systemic vascular resistance was associated with a marked drop in arterial pressure. However, the variation in the pressure drops was greater during total bypass as compared to that prior to bypass. This is explained by the

fact that, on bypass, patients developed different degrees of vasoconstriction which was not present in the patients studied before CPB. It is evident that the hypotensive side-effect of $PGE_1$ is very much dependent on the vasoregulatory state of the patient.

In the third part of our study, when $PGE_1$ was administrated continuously during CPB, severe hypotension occurred mainly in the unstable hemodynamic periods when $PGE_1$ is most likely interacting with the vasoregulation system. A specific antagonist of $PGE_1$ at receptor level is needed to overcome this limiting side effect. Noradrenaline does not seem appropriate because of its $\beta$-adrenergic side-effects. Phenylephrine, which has virtually no $\beta$-adrenergic effects, did not appear adequate in counteracting the $PGE_1$-induced hypotension in our study. Because of this, it was not pssible to administer the dose of $PGE_1$ necessary to obtain an adequate inhibition of platelet aggregation[5].

Opinions about the acceptable minimum blood pressure are still varied. In view of the fact that more and more aged patients undergo open heart surgery, we and other institutions try to maintain the mean blood pressure on CPB at the level of the pre-bypass diastolic arterial pressure and do not accept mean arterial blood pressure drops to below 50 mm Hg. Recently, however, low flow, low pressure ($>$ 30 mm Hg) perfusion has been advocated as a safe procedure in patients undergoing coronary artery surgery[7]. In view of these divergent ideas, no definite indication can be given as to what the safe maximal dosage of $PGE_1$ is, in the light of its hypotensive side effects.

In this respect prostacyclin ($PGI_2$) might be more appropriate because of its stronger inhibition of platelet aggregation without an equivalently stronger vasodilatory effect[6]. However, the experience obtained so far with this drug is mainly in the realm of animal experiments, and results obtained from the latter when using $PGE_1$, have proved invalid when applied to humans. Hemodynamically well-documented studies with $PGI_2$ are needed to show that this strong platelet inhibitor can safely be used in CPB.

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# EPILOGUE

Although the optimal drug for preservation of platelets during cardiopulmonary bypass (CPB) has not yet been developed, some promising indications emerge from this study.

The platelet function inhibiting drugs $PGE_1$ and $PGI_2$ meet the requirements of maintaining a strong platelet function inhibiting effect during infusion, which is immediately reversible. It appeared to be possible to preserve the platelets from damage caused during bubble oxygenator perfusion in dogs and to maintain the hemostatic capacity after perfusion. The platelet damage induced by the bloodgas interface and shear stresses are apparently amenable to improvement by treatment with platelet function inhibiting drugs.

It is also demonstrated that the depressant effect on platelets of protamine hydrochloride, occurring in a critical post-bypass period for the patient, when optimal hemostasis is needed, can be reduced.

Treatment with $PGE_1$ and $PGI_2$ seems to be potentially capable of inhibiting all platelet reactions activated during CPB, which otherwise affect the hemostatic capacity.

Because all basic platelet reactions are triggered during CPB, complete inhibition of all platelet function is needed and not selectively the platelet surface interaction as proposed by Mielke et al.[1]. However, $PGE_1$ and $PGI_2$ appeared particularly in patients to have a pronounced vasodilatory side-effect.

Of the two, $PGI_2$ is in vitro the most potent inhibitor of platelet function and the vasodilating side-effect should therefore be relatively small.

Since 1980, studies have been published on the effect of $PGI_2$ on the platelets during CPB in man (Table 1).

Radegran et al.[2] showed that 20 nanograms of $PGI_2$ per kg per minute did not improve the platelet count. However, he obtained platelet preservation when dosages of 50 nanograms were given. These patients received $PGI_2$ only during the first 30 minutes of bypass. The inhibition of platelet function during this period was effective enough to result in higher platelet counts for the following period. These results accentuate that the events at the start of CPB determine to a large extent the degree of platelet loss. The post-operative blood loss in these patients was slightly less than in the non-treated control group. $PGI_2$ in a dose of 50 nanograms resulted in a significant fall in arterial blood pressure to 26 mm Hg. Although it is mentioned that the post-operative course was uneventful, in general such a perfusion pressure is considered to be unsafe.

Table 1. Studies of $PGI_2$ in clinical cardiopulmonary bypass.

| | n | Dose | Operation | Platelet count | B-TG | $PF_4$ | $TXB_2$ | HSE | $P_{art}$ | Platelet aggregation | Blood loss |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Radegran | 10 | 2-20 | | = | | | | | 43 | | |
| | 12 | 50 | | +* | | | | + | 26* | | — |
| Longmore | 12 | 20 | CABG | +* | | | | | 58 | + | —* |
| Walker | 24 | 20 | CABG(14) | +* | —* | —* | = | | | | |
| | | | VR(7) | + | —* | — | = | | | | — |
| Faichney | 28 | 20 | CABG/VR | +* | —* | —* | = | | 45 | | — (CABG) —* (VR) |
| Fabiani | 2 | 50 | CABG/VR | | | | | | | | |
| | 11 | 25 | | +* | | | | + | 55* | +* | — |

n = number of patients; B-TG: beta-thromboglobulin; $PF_4$: platelet factor 4; $TXB_2$: thromboxane $B_2$; HSE: heparin sparing effect; $P_{art}$: lowest mean arterial pressure; CABG: coronary artery bypass grafting; VR: valve replacement; +: increase; =: no change; —: decrease; *: change is statistically significant.

Longmore et al.[3] treated 12 patients undergoing coronary artery bypass grafting (CABG) with 20 nanograms of $PGI_2$ per kg per minute. He found preservation of platelet number and function with this dose and a significant decrease of post-operative blood loss as well as a reduction in post-operative blood requirements. A heparin sparing effect was demonstrated and the vasodilatation was reported not to be troublesome during CPB.

Walker and Faichney[4 5] published two articles about one study of 28 patients treated with 20 nanograms of $PGI_2$ per kg per minute. Platelet counts were better maintained and a decrease was found in beta-thromboglobulin and platelet factor 4 release in the $PGI_2$ treated group. Furthermore, a reduction of platelet and fibrin deposition on the arterial mesh filter was observed, which might benefit the microcirculation. No statistically significant differences could be found in the extent of bleeding after bypass. However, when the results were studied related to the type of operation a statistically significant reduction of the blood loss of about 400 ml could be found in the $PGI_2$ treated patients undergoing valve replacement. Hypotension was not found to be a problem. An interesting phenomenon in this study was that the greatest fall in platelet count occurred in the first 15 minutes after CPB. This suggests a relation to the administration of protamine. Prolongation of $PGI_2$ until protamine has been given might have preserved the

platelets from the protamine induced damage.

Fabiani et al.[6] treated 2 patients with 50 and 11 patients with 25 nanograms of $PGI_2$ per kg per minute. This resulted in higher platelet numbers and a more depressed platelet function during CPB as compared with a control group of patients. After CPB significantly higher platelet number and function could be measured. The post-operative blood loss was slightly less in the $PGI_2$ treated group. The mean arterial blood pressure in the $PGI_2$ treated group decreased significantly ($p < 0.05$), requiring transfusion and resulting in a decreased urine production. In this study for the first time a statistically significant improvement of platelet aggregation is documented after the $PGI_2$ treatment.

The discrepancies in vasodilating effect of $PGI_2$ in these studies most likely must have been due to differences in hemodynamic condition of the patients. We have documented in our clinical study with $PGE_1$ that the degree of hypotension correlated with status of the systemic vascular resistance (SVR) at the start of the infusion. High SVR resulted in a more severe hypotension. We also investigated the hemodynamic effect of $PGI_2$ during CPB in eight patients. An infusion of 20 nanograms of $PGI_2$ per kg per minute was given during 10 minutes in a hemodynamically stable period. This induced a rapid drop in arterial blood pressure of about 30%, similar to what we have seen with $PGE_1$. It is therefore apparent that $PGI_2$ has at least the same vasodilating effect as $PGE_1$. At these doses the platelet inhibiting effect of $PGI_2$ was not more pronounced than that of $PGE_1$, which is in agreement with our experimental results. This might, however, partly be due to the instability of $PGI_2$.

It can be concluded from these studies, that although $PGI_2$ can potentially preserve platelet number and function during clinical CPB, no significant reduction of post-operative blood loss was obtained in every study. However, its hypotensive side-effect was experienced in most studies. The instability of $PGI_2$ at room temperature is furthermore a limitation for routine use. Recently a stable $PGI_2$ analogue, a carbacyclin derivative (ZK 36374, Schering, Berlin) has been developed. Experimental studies showed an appreciable dissociation between antiplatelet and blood pressure lowering activities of this compound[7]. ZK 36374 might therefore be superior to $PGI_2$ for in vivo use. Further studies are needed to show that this compound can be used to preserve platelets during CPB.

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SUMMARY

# PLATELET PRESERVATION DURING CARDIOPULMONARY BYPASS

The purpose of this thesis was to find an answer to the following problem: does treatment with platelet function inhibiting drugs during cardiopulmonary bypass (CPB) preserve platelet number and function?

In the INTRODUCTION the way in which the coagulation system and the platelets are activated during CPB is described. Heparin, which is routinely given during CPB, inhibits activation of the coagulation system, but does not prevent activation of the platelets. The platelets adhere to the synthetic surfaces and aggregate. Furthermore, general platelet aggregation is induced by shear forces generated in the extracorporeal circuit. All of these factors result in loss of platelet number and function and increase the bleeding tendency after operation. The application of platelet function inhibiting drugs during CPB is only useful if the inhibition is more or less complete, so that the platelets are effectively preserved. The inhibition of platelet function should also be immediately reversible after CPB to obtain a normal hemostasis postoperatively.

In CHAPTER 1, the platelet function inhibiting drugs: Org 4122, Org 4178, sulfinpyrazone, dipyridamole, prostaglandin $E_1$ ($PGE_1$) and prostacyclin ($PGI_2$) were tested in dogs without CPB for their capacity to inhibit platelet function and for the reversibility of this inhibition. $PGE_1$ and $PGI_2$ were the most powerful and best reversible platelet function inhibiting drugs.

In CHAPTER 2, the platelet preserving capacities of $PGE_1$ and $PGI_2$ were compared with a control group of dogs, during bubble oxygenator (BO) perfusion. Treatment with both drugs during CPB resulted in improved platelet number and function thereafter. The post-operative bleeding times, prolonged in the controls, remained normal in the $PGE_1$ and $PGI_2$ treated groups.

In CHAPTER 3, the variables are described which affect platelet number and function in clinical circumstances and which could interfere with the evaluation of platelet function inhibiting drugs. This was studied in patients undergoing coronary artery bypass grafting with comparable perfusion

times. Platelets were better preserved in membrane oxygenator (MO) perfusion when compared to BO perfusion. However, platelet function was also affected by cardiotomy suction and by protamine administration. Patients perfused with the MO had statistically significantly less post-operative blood loss and required fewer blood transfusions when compared to the patients perfused with the BO.

In CHAPTER 4 in a comparable group of patients, perfused with a MO, the platelet preserving capacities of $PGE_1$ were tested. The hypotensive side-effect of $PGE_1$ appeared to be so strong that only very small doses of $PGE_1$ could be given. Treatment with this low dose did not result in preservation of platelet number or function.

In CHAPTER 5, the hemodynamic side-effects of $PGE_1$ were quantitatively described. Treatment with 50 nanogram of $PGE_1$ per kg per minute during the whole period of CPB, was only possible when vasopressors were added, but in most cases the $PGE_1$ dose had to be reduced and in some patients to be stopped to maintain an acceptable perfusion pressure. Infusion of 50 nanogram of $PGE_1$ per kg per minute before CPB induced a fall in blood pressure of 26 mm Hg. When the same dose of $PGE_1$ was given in a hemodynamical stable period CPB, the mean arterial blood pressure dropped from $74 \pm 11$ to $57 \pm 10$ mm Hg. The fall in blood pressure ranged from 0 to 50 mm Hg and was dependent on the peripheral vascular resistance.

In the EPILOGUE a review is given of five studies published on $PGI_2$ treatment during CPB in man. Treatment with $PGI_2$ did preserve platelet number and function and decreased the post-operative blood loss. However, when taking into account our own hemodynamical studies of $PGI_2$ during clinical CPB, we have to conclude that $PGI_2$ still has an unacceptable hypotensive side-effect in CPB in man.

It can be stated that preservation of platelets during CPB can potentially be obtained with platelet function inhibiting drugs like $PGI_2$, but that routine application in open heart surgery will only be feasible if the vasodilatory side-effects can be overcome.

SAMENVATTING

# MEDICAMENTEUZE BESCHERMING VAN THROMBOCYTEN TIJDENS HET GEBRUIK VAN DE HART-LONG MACHINE

In dit proefschrift wordt een onderzoek beschreven met de vraagstelling: leidt behandeling met medicamenten die de thrombocyten-functie remmen tijdens het stromen van bloed door de hart-long machine (HLM), tot beter behoud van thrombocyten aantallen en functie?

In de INLEIDING wordt beschreven dat tijdens het stromen van bloed door de HLM, het stollingssysteem en de thrombocyten geactiveerd worden, zodat het bloed gaat stollen. Deze stolling wordt tegengegaan met heparine, hetgeen echter de activatie van de thrombocyten niet remt. Door de voortdurende activatie verliezen de thrombocyten hun aggregerende functie en de niet meer functionerende thrombocyten verdwijnen uit de circulatie. Dit kan resulteren in een verhoogde bloedingsneiging na de operatie. Om het verlies aan thrombocyten te voorkomen kan het met medicamenten remmen van de thrombocyten-functie zinvol zijn. Deze remming moet dan enerzijds voldoende sterk zijn om de sterke aktivatie tijdens de operatie te voorkomen en anderzijds onmiddelijk na de operatie kunnen worden opgeheven, om een normale bloedstolling te herstellen.

HOOFDSTUK 1 beschrijft het onderzoek naar de mate van remming van de thrombocytenfunctie en het herstel hiervan na toediening van: Org 4122, Org 4178, sulfinpyrazon, dipyridamol, prostaglandine $E_1$ ($PGE_1$) en prostacycline ($PGI_2$). Dit onderzoek werd verricht bij honden. Hierbij bleek dat $PGE_1$ en $PGI_2$ de meest krachtige thrombocyten-functie remmende medicamenten zijn, waarbij tevens dit remmende effect na stoppen van de toediening direct was op te heffen.

HOOFDSTUK 2 beschrijft het onderzoek bij honden naar de beschermende werking van $PGE_1$ en $PGI_2$ op de thrombocyten tijdens het gebruik van een bubble oxygenator (BO) in de HLM. Het bleek mogelijk met beide medicamenten de thrombocyten in aantal en functie beter te behouden dan zonder deze medicamenten. De bloedingstijden bleven na de operatie normaal in tegenstelling tot die van de niet behandelde honden, waarbij de bloedingstijden sterk verlengd waren.

HOOFDSTUK 3 geeft het onderzoek weer naar de variabelen die bij patiënten invloed kunnen hebben op het aantal en de functie van de thrombocyten en die het effect van thrombocyten-functie remmende medicamenten zouden kunnen beïnvloeden. Dit werd onderzocht bij patiënten die een kransslagader operatie ondergingen en waarbij gedurende een vergelijkbare periode van de HLM gebruik gemaakt werd. Tijdens het gebruik van een membraan oxygenator (MO) in de HLM, werd de thrombocyten-functie minder beschadigd dan tijdens het gebruik van een BO. De thrombocyten-functie die tijdens het gebruik van de MO beter bewaard bleef, werd echter in sterke mate aangetast door het zuigsysteem dat gebruikte werd om verloren gegaan bloed terug te brengen in de HLM. Ook het geven van protamine, ter neutralisatie van heparine aan het eind van de operatie, had een nadelige invloed op de thrombocyten-functie. Ondanks deze nadelige effecten was er toch na het gebruik van de MO minder bloedverlies, waardoor ook minder donorbloed gegeven hoefde te worden.
HOOFDSTUK 4 beschrijft het onderzoek naar het vermogen van $PGE_1$ om de beschadiging van thrombocyten tijdens kransslagader operaties te verminderen. Bij deze patiënten bleek $PGE_1$ een veel sterkere bloeddruk-verlagende bijwerking te hebben dan bij de proefdieren. Hierdoor konden minder hoge doseringen gegeven worden, zodat de thrombocyten-functie onvoldoende geremd kon worden. Er was dan ook weinig verschil aantoonbaar in het effect op de thrombocyten in vergelijking met de onbehandelde groep patiënten. De bloedingsneiging was gelijk in beide groepen.
In HOOFDSTUK 5 wordt nader ingegaan op de bijwerkingen van $PGE_1$ op de bloeddruk. Als geprobeerd werd 50 nanogram $PGE_1$ per kg per minuut gedurende de gehele procedure te geven, traden er perioden op waarin de bloeddruk zo laag werd, dat bloeddrukverhogende middelen nodig waren. Bij een aantal patiënten moest de dosering van $PGE_1$ worden verminderd en bij sommigen zelfs gestopt. Indien $PGE_1$ gegeven werd in de periode voordat de HLM was aangesloten, bleek een bloeddrukdaling van ongeveer 26 mm Hg op te treden. Als het $PGE_1$ kortdurend werd gegeven, tijdens een stabiele periode van de procedure, bleek de mate van bloeddrukdaling sterk afhankelijk van de perifere vaatweerstand.
In de EPILOOG worden de vijf tot nu toe gepubliceerde klinische studies, betreffende de toepassing van $PGI_2$ tijdens het gebruik van de HLM, besproken. Het bleek mogelijk een beter behoud van de thrombocyten aantallen te bewerkstelligen en de thrombocyten-functie te preserveren, hetgeen resulteerde in een daling van het bloedverlies na de operatie. Echter onze eigen studie, waarin het effect van $PGI_2$ op de bloeddruk tijdens open-hart

operaties bij patienten werd gemeten, toonde aan dat ook $PGI_2$ een sterke bloeddruk-verlagende bijwerking heeft.

Concluderend: remming van de functie van de thrombocyten, tijdens het stromen van bloed door de HLM, kan inderdaad leiden tot een beter behoud van de thrombocyten aantallen en functie. De bloeddruk-verlagende bijwerking van $PGE_1$ en $PGI_2$ beperkt vooralsnog de routinematige toepassing in de open-hart chirurgie.

# CURRICULUM VITAE

Jan van den Dungen was born on July 30th, 1951 in Oldenzaal, The Netherlands.
After graduating in 1969 from high school (HBS-B) in Enschede, he studied medicine at the University of Groningen and completed his studies in 1976.
In 1977, he was enlisted into the Royal Dutch Army, where as Medical Officer he was given the opportunity to undertake a research project at the Department of Experimental Surgery (Chairman: Prof. Dr. Ch. R. H. Wildevuur) in the Department of Surgery (Chairman: Prof. Dr. P. J. Kuijjer) of the University of Groningen.
Since 1979, he has been employed as surgical resident in the Department of Surgery, of the University Hospital, Groningen.